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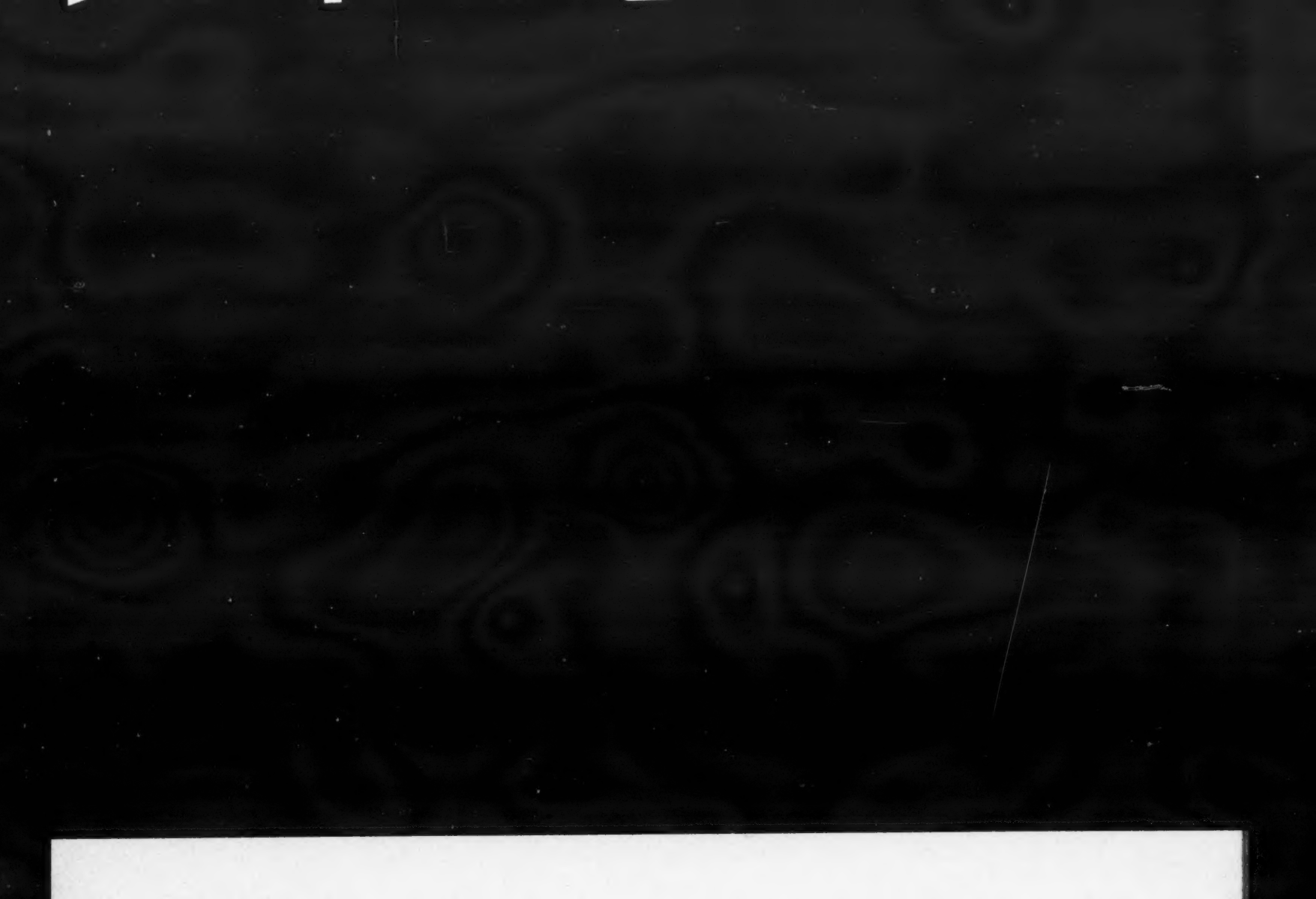

Canadian Journal of Botany

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VOLUME 37 MAY 1959 NUMBER 3

CHROMOSOME MORPHOLOGY IN THE GENUS AVENA[1]

T. RAJHATHY AND J. W. MORRISON

Abstract

The chromosomes of the *Avena* species were studied and their karyotypes established. The karyotype of the diploid species (excluding *A. longiglumis*) is designated as A. *Longiglumis* has a different karyotype with at least three chromosomes that have major changes. This karyotype is a modified A, designated as A′. The designation of the tetraploid karyotype is AB, including the basic set of chromosomes A and a second completely different set B. In the hexaploid species the basic set A is present but the B set is not; therefore the proposed designation is ACD. These results indicate an alloploid origin for the polyploid species.

Introduction

The cytology and genetics of oats and its relatives have received less attention than the other major cereals. The chromosome make-up of the genus is similar to that of wheat. The three series are based on the following chromosome numbers: diploids $2x = 14$, tetraploids $4x = 28$, and hexaploids $6x = 42$(3). On the basis of meiotic studies, Nishiyama (12, 13, 14) designated the genomes of *A. strigosa* as A, of *A. barbata* as AB′, and of *A. fatua* as ABC. Other studies (see Sampson (17)) have thrown some doubt on the existence of the B genome in the hexaploid species. The studies of Ellison (2), Jones (8), and Zillinsky (19) have also contributed some information about interspecific crosses in *Avena*. There are no reports of intergeneric crosses, consequently the related genera are not known (4, 7). It is not yet certain whether the polyploid species are autoploids or alloploids.

Along with the scarcity of results in *Avena* the morphology of the chromosomes has received little attention and the karyotypes of the oat species are not established. There are only two previous reports on oat chromosomes. Oinuma (16) studied the chromosomes of *A. strigosa*, *A. barbata*, *A. byzantina*, *A. fatua*, and two varieties of *A. sativa*. He described the chromosomes of *A. strigosa* as the standard set. According to him, *A. barbata* with 28 chromosomes has the doubled set of the diploid *A. strigosa*, and the hexaploid species have more or less a tripling of the standard set. Nishiyama and Ikuzawa (15) briefly reported on the chromosomes of *A. strigosa*, *A. brevis*, *A. wiestii*, and *A. nudibrevis*.

The present report describes the morphology of oat chromosomes, giving the karyotypes of the different species. This is the first step in a study of the relationship and evolution of the genus *Avena* and its relatives.

[1]Manuscript received November 24, 1958.
Contribution from the Cereal Crops Division (No. 239), Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ontario.

[The previous number of Can. J. Botany (**37**, 169–329) was issued March 19, 1959.]

Methods

Seeds were germinated at room temperature on filter paper in Petri dishes. Roots 10–15 mm long were pretreated with monobromonaphthalene to facilitate preparation. The roots were fixed with La Cour's fluid, 2BD, and after hydrolyzation stained with Feulgen. The procedure used is described by Morrison (11). All studies have been made from metaphases and also some prophases of mitosis in root tips.

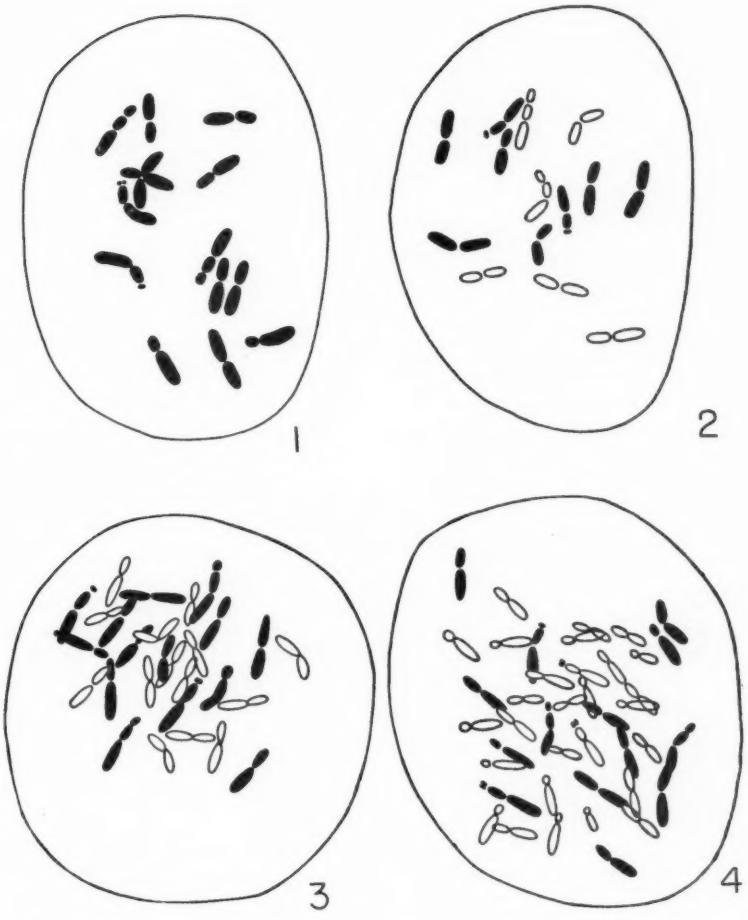
In the description of chromosomes those having a constriction subtending a nucleolus-organizing region, i.e., having a satellite, are called sat.-chromosomes; those with the centromere in a median, submedian, etc., location are given the shorter term.

Material

Most of the samples of the species studied were obtained by our oat unit principally from Iowa, Wales, and Uruguay. The species studied are grown every year in this division.

Species	Received from
Diploids	
A. longiglumis C.D. 6080	Aberystwyth, Wales
A. longiglumis C.D. 7567	Berlin-Dahlem, Germany
A. longiglumis C.D. 7568	Delft, Holland
A. hirtula C.D. 7569	La Estanzuela, Uruguay
A. strigosa C.D. 3820	La Estanzuela, Uruguay
A. strigosa L. v. *typica* Vav. C.D. 7570	Iowa, U.S.A.
A. brevis C. D. 7571	
A. brevis Roth v. *semiglabra* Malz. C.D. 7572	Iowa, U.S.A.
*A. barbata** C.D. 6572	Portugal
Tetraploids	
A. barbata C.D. 7575	California
A. vaviloviana (Malz.) Mordv. v. *pseudo-abyssinica* Malz. C.D. 7310	Leningrad, U.S.S.R.
A. vaviloviana (Malz.) Mordv. v. *pilosiuscula* Malz. C.D. 7311	Leningrad, U.S.S.R.
A. abyssinica C.D. 4549	U.S.D.A.
A. abyssinica C.D. 4550	U.S.D.A.
A. strigosa autotetraploid C.D. 3820[2] produced by F. J. Zillinsky	
A. strigosa autotetraploid C.D. 7574 produced by I. Nishiyama	
Hexaploids	
A. sterilis ssp. *ludoviciana* C.D. 1794	Oslo, Norway
A. sterilis v. *maxima* C.D. 7575	
A. sterilis C.D. 7576	Greece
A. fatua C.D. 7577	
A. byzantina C.D. 6614	Uppsala, Sweden
A. sativa cv. Victory C.A.N. 519	
Garry C.A.N. 699	
Rodney C.A.N. 761	
Lanark C.A.N. 541	
Vigor C.D. 7181	

*This diploid species is an interesting form received from Portugal as *A. barbata*. The development and external morphology are very similar to the tetraploid form. Both have the same prostrate growth habit, late maturity, similar shape of spikelets, type of articulation, and pubescence. We have retained this as *A. barbata* diploid form. Malzew (10) referred to the existence of a diploid *A. barbata*.



FIGS. 1–4. Chromosome drawings in *Avena* root tip, ×1200. Fig. 1. *A. strigosa*, the basic set of chromosomes. Fig. 2. *A. longiglumis*, the chromosomes in white are involved in major changes. Fig. 3. *A. abyssinica*, the basic set is in solid black. Fig. 4. *A. sativa* (Victory), the basic set is given in solid black.

Results

Diploid Species

All the species studied except *A. longiglumis* have the same karyotype. The basic set of chromosomes is that of *A. strigosa*, *A. hirtula*, *A. wiestii*, *A. brevis*, and diploid *A. barbata* C.D. 6572 (Figs. 1, 5).

The karyotype of the diploid species is composed of a long submedian chromosome with a small satellite attached to the short arm; a slightly shorter sat.-chromosome with a large satellite attached to the shorter arm (the ratio of the short arm:satellite is about 2:1); a subterminal with a very short arm (ratio about 4:1); a median, being the largest chromosome; and three submedians with an approximate arm ratio of 3:2. They are shorter than the previous chromosomes.

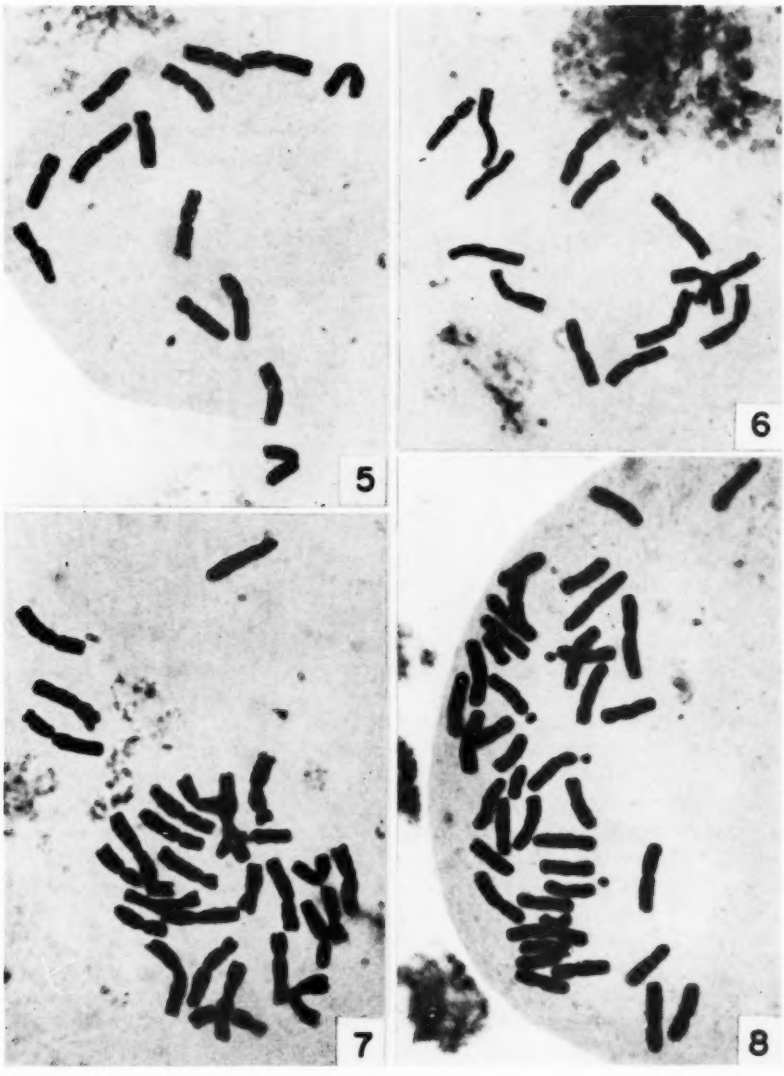
A. longiglumis has a different karyotype. All samples studied have the same karyotype (Figs. 2, 6, 9*b*). There is no difference in the sat.-chromosome with the small satellite; the other sat.-chromosome is slightly longer than the corresponding one in the basic set, and the ratio of the short arm:satellite is about 1:1 instead of 2:1. *The subterminal chromosome of the basic set is not present.* There are two pairs of long median chromosomes: one is an alien chromosome not present in the basic set. There are the usual two submedians which seem to be unchanged from the basic set. The remaining chromosome is median and has a length between that of the two submedians. Four chromosomes are apparently unchanged.

Tetraploid Species

The tetraploid species *A. barbata*, *A. vaviloviana*, and *A. abyssinica* have the same karyotype. The basic set is definitely present in the tetraploids as shown in Figs. 3, 7, 10*a*. The second set does not contain any chromosomes that are especially noticeable. In it three groups were established: (*a*) four median or near median chromosomes, (*b*) two long submedians, and (*c*) one short submedian chromosome. The autotetraploid *A. strigosa* has, as expected, the basic set doubled.

Hexaploid Species

All the hexaploid species studied have the same karyotype. Out of 21 chromosomes seven are the familiar basic set. The rest of the chromosomes were placed in six groups (Figs. 4, 8, 10*b*). There is a sat.-chromosome with submedian centromere and with a small satellite attached to the short arm. This chromosome is very much like the sat.-chromosome with the small satellite in the basic set, but is slightly shorter. The second group contains five long subterminals which are similar to the subterminal of the basic set. The third group is made up of one subterminal chromosome which is the shortest in the genus—about one third of the longest chromosome. The fourth group is formed by two submedians, one longer than the other. In the fifth group there are two long medians and in the sixth, three short median chromosomes.



FIGS. 5–8. Root-tip metaphases in *Avena* species, ×1600. Fig. 5. *A. strigosa*, note the sat.-chromosomes and the subterminals. Fig. 6. *A. longiglumis*, note the size and short arm:satellite ratio in the chromosomes with large satellite, and the lack of subterminals. Fig. 7. *A. vaviloviana*, note the number of sat.-chromosomes and subterminals. Fig. 8. *A. sterilis*, note the sat.-chromosomes, the 12 long and 2 extremely short subterminals.

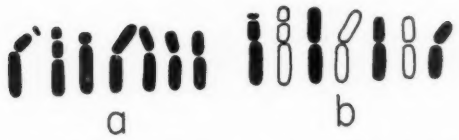


FIG. 9. Karyograms of the diploid *Avena* species, ×1700. (*a*) Karyotype A. (*b*) Karyotype A′.

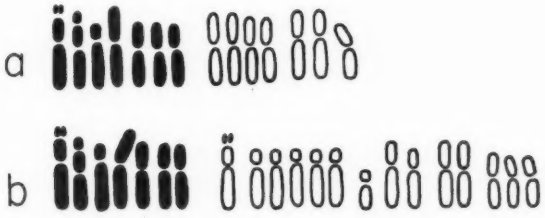


FIG. 10. Karyograms of the tetraploid and hexaploid *Avena* species, ×1700. (*a*) Karyotype AB. (*b*) Karyotype ACD.

Discussion

As mentioned in the Introduction there are two reports on *Avena* chromosomes. Our observations on the diploid species agree with the description of Nishiyama and Ikuzawa (15). Oinuma (16) gave a similar description for the chromosomes of *A. strigosa* but we did not observe the secondary constriction of the subterminal. Our basic set can safely be regarded as genome A. No one has studied the karyotype of *A. longiglumis*. It can be supposed that even this species has the basic karyotype like the other diploids, but the chromosomes have undergone major changes in the course of evolution. As shown in Figs. 2, 6, and 9*b* three chromosomes are involved in these changes. Minor changes involving small segments might not appear. Substantiating the change in karyotype of this species is evidence from meiotic studies of hybrids with *A. longiglumis*. In the hybrids, chains of four, six, and sometimes eight chromosomes are involved (6). The mean of six would support our assumption that three chromosomes are affected by interchanges. Sampson (17), referring to Ellison's examination of *A. longiglumis* × *A. abyssinica* F_1 hybrids (2), stated that it is not clear whether *A. longiglumis* is a modified genome A or a modified B. Comparing the chromosome morphology of the basic set A with the second set of the tetraploids B, it is quite obvious that *A. longiglumis* has a modified A karyotype which can be designated as A′.

The colchicine-induced autotetraploid *A. strigosa* shows, as expected, the doubled basic set AA without any visible structural changes. The natural tetraploid species, *A. barbata*, *A. abyssinica*, and *A. vaviloviana*, are different. In these species the number of sat.-chromosomes and subterminals are just the same as in the diploids. The basic set A is present. The second set

does not contribute any chromosomes with a distinct structure (Fig. 10*a*), not even another sat.-chromosome. This karyotype is designated as B, consequently the karyotype formula of the tetraploids is AB.

The karyotype analysis does not support the hypothesis of autoploid origin of the tetraploid species as supposed by Oinuma (16), and Brücher (1). On the contrary, it indicates an alloploid origin. Our findings disagree with Oinuma, who claimed that *A. barbata* has the doubled set of *strigosa*.

Our results agree with the observations of Nishiyama (12). At meiosis in F_1 hybrids of *A. barbata* × *A. strigosa* usually 7^{II} including trivalents, but sometimes $8–9^{II}$, were found. Later Nishiyama (13) reported 12^{II}, sometimes 13^{II}, in hybrids of *A. strigosa* ($4x=28$) × *A. barbata* while the number of univalents varied between 0 and 5. This would indicate that the genome A of *barbata* is completely homologous with the *strigosa* genome A while the second genome B is partially homologous with the A. On this basis Nishiyama gave the designation AB′. The higher number of bivalents may also be due to autosyndetic pairing. The autotetraploids and their hybrids need further study.

As can be seen in Figs. 4, 8, and 10*b* the basic set A is also present in the hexaploid species. Besides those of the basic set, there is another sat.-chromosome. What is perhaps the most striking feature of the hexaploid karyotype is six subterminal chromosomes. The B set has none. The three fairly short medians are not present in the B set. It is therefore quite obvious from this study that the B set of the tetraploids is not present in the hexaploids. As the remaining 28 chromosomes are not a duplication of one set they must be composed of two genomes and thus the hexaploid karyotype must be ACD.

Again, our results disagree with Oinuma (16), who found in hexaploids the triplicate of the *strigosa* set A showing only very minor changes, some secondary constrictions.

The data from pairing studies in pentaploids are conflicting but in general the genome designation of ACD is supported. Emme (5) showed $7–9^{II}$ in *A. sativa* × *A. abyssinica* and *A. barbata*. Spier (18) in hybrids of *A. abyssinica* × *A. sterilis* obtained $7–11^{II}$ and $13–23^{I}$. In a hybrid of *A. sativa* × *A. strigosa* Kihara and Nishiyama (9) reported 7^{II} with a range of $3–9^{II}$. These data prove that the genome A is present in the tetraploids and in the hexaploids as well. They also suggest that genome B is not present in the hexaploids. However, Nishiyama (12) found in F_1 hybrids of *A. barbata* × *A. sterilis* $7–13^{II}$ and in *A. barbata* × *A. fatua* $2–11^{II}$ and from this indication of partial homology concluded that the B genome of the tetraploids was the second genome of the hexaploids. This would validate his genome designation, ABC given for *A. fatua*. Only a few cells were found with the higher number of bivalents. The mean number was less than seven and nearly all the bivalents were rod bivalents that might have been formed by pairing of homoeologous segments or autosyndetic pairing. This pairing is common in most haploids. Thus the genome designation based only on meiotic pairing is not certain. From our observation of the karyotype we conclude that the hexaploid genomes should be ACD.

Although the alloploid origin of the polyploid species is obvious, the sources of genomes B and CD are still unknown. The build-up of hexaploids could occur in one step (A + CD) or in two steps (A + C + D). In order to obtain information about the origin and evolution of *Avena* species an investigation of the Aveneae tribe is unavoidable. In oats only one genome, the basic set, is common to every species. Hence the pattern of segregation in interspecific hybrids will differ from that of pentaploid wheat hybrids and this, too, requires our attention.

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STUDIES ON SLIME ACCUMULATIONS IN PULP AND PAPER MILLS

II. PHYSIOLOGICAL STUDIES OF PHIALOPHORA FASTIGIATA AND P. RICHARDSIAE[1]

D. BREWER

Abstract

Physiological studies of six isolates of *Phialophora fastigiata* and two isolates of *P. richardsiae* from pulp mills have been made to determine conditions for growth. Isolates of *P. fastigiata* grew from at about 3° C to 30–35° C, with optimum conditions at 20 to 25° C and pH 4 to 9. The temperatures for *P. richardsiae* were somewhat higher. No vitamins were required by *P. fastigiata* in a basal glucose mineral medium with ammonium tartrate. Ability to metabolize 11 carbohydrates as monosaccharides and oligosaccharides, but not D-arabinose, has been demonstrated. Likewise 16 sources of nitrogen as ammonia, nitrate, and various amino acids, but not cysteine, were utilized unequally. The differences between isolates of *P. fastigiata* from the same slime were as great as between isolates from different mills.

Introduction

The growth of both fungi and bacteria in pulp and paper mills forms a slime which is of both scientific and practical importance. *Phialophora fastigiata* (Lagerb. and Melin) Conant was the most widespread of all the fungi that were isolated from pulp and paper mills in Newfoundland and New Brunswick (1) and has since been found to be common in mills in Quebec and Ontario. This fungus thus appears to be of special importance in the accumulation of slime and it was thought that physiological studies of isolates might reveal some differences between them to account for the diversity of the locations of their occurrence. *Phialophora richardsiae* (Nannf.) Conant also occurred quite commonly in slime and two isolates of this fungus were included in these studies.

Experimental

Materials

The isolates employed in these studies were obtained from six samples of slime from four mills in New Brunswick and Newfoundland.

Isolates 4-3D and 4-3F were from the same sample, as were 5-4A and 5-4J. Isolates 3-7H, 3-5F, and 3-8E were from the same mill, but from different samples.

[1]Manuscript received January 2, 1959.
Contribution from National Research Council of Canada, Atlantic Regional Laboratory, Halifax, Nova Scotia.
Issued as N.R.C. No. 5086.

Isolate	Species	Location of accumulation in the mill	Temperature at place of accumulation
1-2E	*Phialophora fastigiata*	Sulphite stock screens	7° C
4-3D	*Phialophora fastigiata*	Sulphite flat screens	7° C
4-3F	*Phialophora fastigiata*	Sulphite flat screens	7° C
3-7H	*Phialophora fastigiata*	Head box	30° C
5-4A	*Phialophora fastigiata*	Sulphite seal pit	3.5° C
5-4J	*Phialophora fastigiata*	Sulphite seal pit	3.5° C
3-5F	*Phialophora richardsiae*	Machine wire pit	29.5° C
3-8E	*Phialophora richardsiae*	Broke sump	28° C

Temperature Studies

The isolates were inoculated in duplicate on potato dextrose agar and incubated at 3°, 10°, 15°, 20°, 25°, 30°, and 35° C. The rates of growth of the isolates at the different temperatures were determined from the average increase in the diameter of the culture during the 5-day period after a 5-day incubation period. A 10-day incubation period was allowed at 3° and 10° C.

Isolates of *P. fastigiata* differed in the temperature for optimum growth. Thus for isolates 4-3D, 4-3F, and 3-7H it was 20° C, for isolate 1-2E it was between 20° and 25° C, and for isolates 5-4A and 5-4J, 25° C. The minimum temperature for growth was not determined exactly, but it was certainly below 3° C at which temperature the isolates grew very slowly. Above 25° C there was a sudden drop in the growth rate, only trace amounts occurred at 30° C, and no growth at 35° C.

The isolates of *P. richardsiae* had a higher optimum, 30° C, a higher minimum, between 10° and 15° C, and a higher maximum, above 35° C. This is to be expected, since they were isolated from slimes accumulating at higher temperatures than were the slimes from which all but one of the isolates of *P. fastigiata* were obtained. The exception, 3-7H, was isolated from the same mill as the two isolates of *P. richardsiae* and from a slime accumulating at a similar temperature.

pH Studies

The organisms were grown at 25° C on potato dextrose agar adjusted, after autoclaving, to various pH levels ranging from pH 2.9 to pH 9. The rate of growth was measured as before. The isolates grew at all levels of pH and the rate of growth was practically uniform throughout the range pH 4 to 9, except in the case of the two isolates of *P. richardsiae* when at pH 4 it was reduced to two thirds of the rate at pH 5.

Vitamin Requirements

The basic medium was a modification of that suggested by Foster (2) for the study of biochemical mutants of *Neurospora* and had the following composition.

Ammonium tartrate	4.6 g
Magnesium sulphate (hydrated)	0.5 g
Potassium dihydrogen phosphate	1.0 g
Calcium chloride	0.1 g
Sodium chloride	0.1 g
Iron	0.2 mg
Boron	0.01 mg
Copper	0.1 mg
Manganese	0.02 mg
Molybdenum	0.02 mg
Zinc	2.0 mg
Glucose	20.0 g
Distilled water	1 liter

It was found that all the isolates grew well without the addition of any growth substance to the medium, except the two isolates of *P. richardsiae*, which required the addition of biotin.

Utilization of Carbohydrates

The basic medium was that used in the experiments on vitamin requirement with the ammonium tartrate replaced by 5.25 g of potassium nitrate per liter. The solutions of the carbohydrates were autoclaved separately and mixed with the other components when cool to establish a concentration of 1%.

The inoculum was a disk, 4 mm in diameter, taken from a culture on 2% malt agar. The isolates were grown in the dark in still culture in 25 ml of the nutrient medium at 25° C for 10 days.

The mycelium was obtained by filtering the culture through cloth and the weight of the mycelium dried at 105° C was taken as the measure of growth. Table I lists the averaged weights of ovendry mycelium from duplicate cultures of each isolate grown in each medium.

With the exception of D-arabinose, all carbohydrates were utilized by all isolates, although at different rates. It was found that not only the isolates of *P. fastigiata* from different mills but also isolates of this species from the same sample of slime varied in their abilities to utilize different carbon sources. The two isolates of *P. richardsiae* were quite similar in the amount of growth produced by each in the different media.

TABLE I

Averaged weights of ovendry mycelium from duplicate flasks, in milligrams

Carbon source	Isolate							
	1-2E	4-3D	4-3F	5-4A	5-4J	3-7H	3-5F	3-8E
D-Glucose	98	65	88	57	82	72	83	100
D-Galactose	104	68	50	45	65	111	53	42
D-Mannose	67	73	53	52	58	109	111	104
D-Fructose	32	13	18	10	15	27	132	114
D-Xylose	98	65	62	56	64	95	121	113
D-Arabinose	0	0	0	0	0	0	0	0
L-Arabinose	17	40	13	trace	15	12	63	85
Sucrose	84	60	63	49	60	66	107	96
Maltose	59	57	25	30	29	79	65	90
Cellobiose	107	75	32	26	27	93	117	101
Raffinose	67	85	32	41	85	82	23	23

Utilization of Nitrogenous Compounds

The basic medium was again the same. The carbon source was 2% glucose and the nitrogen source was incorporated into the medium to give 0.35 g N per liter. Table II lists the averaged weights of oven-dry mycelium from triplicate cultures of each isolate grown in each medium for 14 days.

As in the studies on carbohydrate utilization, the variation amongst the isolates of *P. fastigiata* was apparent not only between those obtained from different mills but also between those from the same sample. The two isolates of *P. richardsiae* were similar in their ability to utilize the various nitrogen sources.

TABLE II

Averaged weights of ovendry mycelium from triplicate flasks, in milligrams

Nitrogen source	Isolate							
	1-2E	4-3D	4-3F	5-4A	5-4J	3-7H	3-5F	3-8E
Ammonium tartrate	155	129	159	199	186	161	158	152
Ammonium sulphate	66	39	49	59	45	47	92	91
Potassium nitrate	195	171	197	168	192	189	174	163
L-Asparagine	242	220	212	228	231	149	156	143
D-Aspartic acid	215	123	105	50	53	203	137	140
β-Alanine	127	19	80	96	53	41	170	166
L-Arginine monohydrochloride	140	112	126	132	122	136	143	140
L-Cysteine hydrochloride	0	0	0	0	0	0	0	0
L-Glutamic acid	166	137	147	96	126	221	148	153
Glycine	162	39	249	236	232	211	41	33
DL-Isoleucine	82	121	83	56	113	95	67	66
L-Proline	234	233	216	253	252	201	173	176
DL-Serine	50	trace	108	38	150	207	97	95
DL-Tryptophane	42	53	45	35	35	77	103	104
DL-Valine	71	194	132	71	130	183	127	131
L-Ornithine hydrochloride	113	100	90	110	106	138	115	127

Discussion

Except for one isolate, the optimum temperatures for growth of isolates of *Phialophora fastigiata* were well above the temperatures at which they had been growing in the mills. The isolates from slime accumulating at the lowest temperature had the highest optimum. The growth of all isolates at the accumulating temperatures was very slow, yet the growth under natural conditions was rapid. The effect of temperature on the selection of species and strains is not directly apparent from this type of study, as it is necessary to consider the flora as a whole. The results in these experiments indicate that temperature had little effect on which strains of the organism would be selected at certain locations, yet it is known that a change in the temperature at the point of accumulation can radically change the composition of the slime.

The isolates of *Phialophora fastigiata* employed in these studies were obtained from a wide variety of sources, where the available nutrients could have differed quite considerably. Isolate 1-2E was obtained from a sulphite mill system, as were isolates 4-3D and 4-3F. Isolates 5-4A, 5-4J,

3-7H, and the two isolates of *P. richardsiae* were obtained from newsprint mills where the sulphite and groundwood systems are not entirely separate. Mill No. 3 differed from mill No. 5 in that there was a smaller amount of recirculation of the white water. Considering the possible differences in the nutrient supply, it could be expected that the isolates from different mills might differ in their abilities to utilize various carbohydrates and nitrogen sources. However, the results of the present investigations showed that there could be as great a difference between isolates from the same slime accumulation as between isolates from different mills.

The range of carbohydrates and nitrogenous compounds utilized, together with the wide range of pH values favorable to good growth, suggests why *P. fastigiata* is common in different types of mills. However, the isolates studied do not differ in any spectacular way from other filamentous fungi regarding their abilities to utilize various nutrient sources, or in their pH and temperature requirements. It may be that further studies, when more is known regarding the 'natural' substrate, will explain the selection of fungi capable of rapid and competitive growth under the conditions existing in a pulp mill.

In these studies there has been no consideration of the effects of fungal and of fungal and bacterial associations, which could possibly play influential roles in the selection of organisms. The effect of the physical environment should also be considered, since this is a factor that can vary considerably from one location to another.

Acknowledgments

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A NOTE ON SPOROGENESES IN DIPCADI SEROTINUM (L.) MEDIC.[1]

M. S. CHENNAVEERAIAH[2] AND T. S. MAHABALE[3]

Abstract

Distinguished from the rest of the genera of the Scilloideae, *Dipcadi* is characterized by tuberous scapigerous herbs with racemed flowers, cylindric perianth of six erect segments, the outer recurved from about the middle and the inner at the tips only, included stamens and a short capsule which is broad, tridymous, loculicidal, and many-seeded. The genus is insufficiently known both cytologically and embryologically. The present paper gives an account of micro- and mega-sporogenesis in a plant of *D. serotinum*. This taxon, with a diploid chromosome number of eight and with all chromosomes having satellites and all apparently taking part in nucleolar organization, has other features of interest such as meiotic irregularities, abnormal megaspore tetrads, and polyspory. These have been recorded here and explanations are given as far as possible.

Introduction

The Liliaceae have always been favorites for cytological and embryological studies. In spite of the literature accumulated on it, the study of this family is far from complete. Schnarf (16) has reviewed the embryological literature on this family up to the year 1930. After that, a number of important publications have appeared revealing many interesting features. Different members of the family are shown to have five distinct types of embyro sac development, viz., *Polygonum*, *Allium*, *Drusa*, *Fritillaria*, and *Adoxa* types. Eunus (7) has given a full review of these. Of late the subfamily Scilloideae has been receiving keener attention from both the embryological and the taxonomic points of view (cf. 6, 18). However, the genus *Dipcadi* in this subfamily has remained inadequately studied, perhaps due to difficulty in procuring the material. Cytological studies for some of the species have been made by Levan (11), Battaglia (3), and Mahabale and Chennaveeraiah (12). The only embryological work is by Buchner (5) for the species *D. serotinum*. Her findings include a brief description of the style and stigma, septal nectaries, and megasporogenesis. She did not, however, study microsporogenesis or other aspects in this or other species of the genus. The present paper gives certain interesting observations made in a plant of *D. serotinum*, whereas extensive embryological work on other species of the genus will appear elsewhere. The material for the present study consisted of an inflorescence fixed in formalin–acetic–alcohol by the junior author at 'Hortus Botanicus', Amsterdam. Even the scanty material available to us revealed many noteworthy features which would otherwise have remained obscure.

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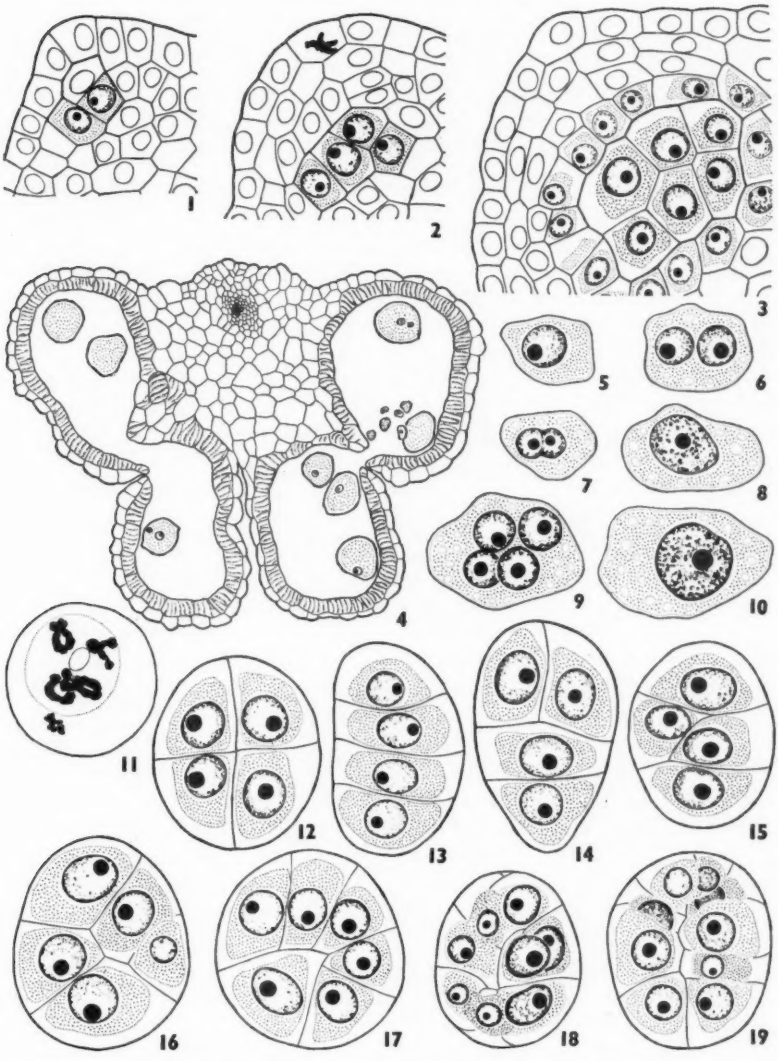
Microsporangium and Microsporogenesis

At each corner of the young anther one to three hypodermal cells differentiate as the archesporial cells (Fig. 1). The archesporial cell first cuts off an outer primary parietal cell and an inner primary sporogenous cell. The primary parietal layer divides to form another layer and the latter divides to form a third one (Figs. 2, 3). Thus in the four-layered wall of the anther, the innermost one forms the tapetum while the other three of the periphery form the epidermis, endothecial, and middle layers respectively (Fig. 3). At maturity the microsporangia of each side of the anther become confluent owing to the breakdown of the partition between them. A stomium is organized during dehiscence and the hypodermal layer develops into the fibrous endothecium (Fig. 4).

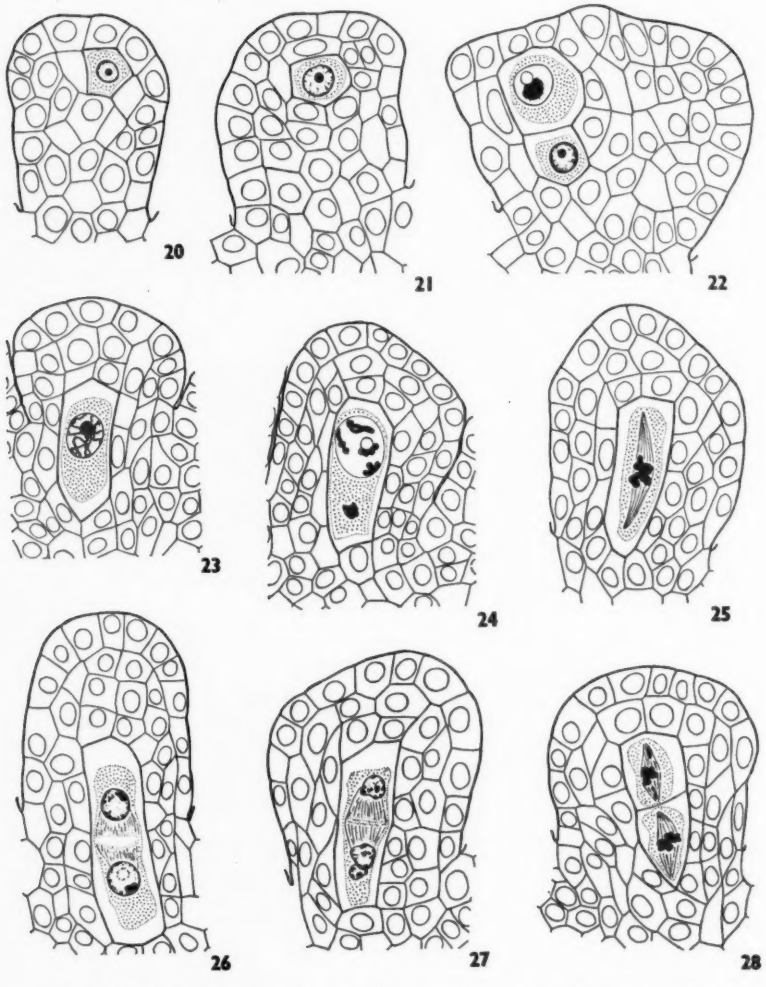
The tapetal cells at the beginning are uninucleate (Fig. 5). They undergo typical mitotic divisions and become binucleate (Fig. 6). Sometimes the two nuclei may fuse resulting in a secondary uninucleate condition or the two nuclei may divide again resulting in a four-nucleate condition (Figs. 7–9). A large polyploid nucleus is sometimes formed by the fusion of all these nuclei (Fig. 10). The tapetum starts degenerating when the microspores are developing and is of the secretory type.

The microspore mother cells become rounded before they begin to divide. They pass through a typical synizetic knot during meiotic division. Figure 11 shows diakinesis in which four bivalents are seen. Apart from these, a heavily stained body which seems to be chromosomal is seen outside the nuclear membrane. The origin of this heavily stained body could not be traced due to insufficiency of the material. The division of the microspore mother cell is of the successive type as in the other species of the genus. The microspore tetrads that are formed are mostly isobilateral, although linear, T-shaped, and decussate types are also found (Figs. 12–15).

Not infrequently more than four microspores are formed from a spore mother cell. Such a condition of polyspory or polyad is not reported for this species previously nor does it occur in the other species of the genus investigated. A pentad, hexad, octad, and one with nine spores were observed by us. In these cases all the spores and their nuclei are not of the same size. Figure 16 is that of a pentad with four spores having the nuclei more or less of the same size, but in the fifth one it is slightly smaller. In a hexad, represented in Fig. 17, the nuclei of four spores are of one size and those of the other two are of another size. In the octad observed, four spores have the nuclei of the normal size whereas the other four spores have slightly smaller ones (Fig. 18). In the one where nine spores are seen, four have nuclei of the same size but those of the others are of different sizes (Fig. 19). It is noticeable that in each of the above cases of polyspory four spores have nuclei of the same size. It is very difficult to account for all these irregularities without a thorough study. The chromatin-like extra body, seen at diakinesis, which perhaps resulted from a division of an additional nucleus that might have been



FIGS. 1–19. Microsporangium and microsporogenesis of *Dipcadi serotinum*. Magnification: 1–3, ×638; 4, ×95; 5–19, ×1130. Fig. 1. Anther lobe with archesporial cells. Fig. 2. Anther lobe with sporogenous cells and two parietal layers. Fig. 3. Anther lobe showing the innermost layer of the four-layered wall differentiated as tapetum. Fig. 4. Transverse section of anther showing confluent loculi, fibrous endothecium, and sterile pollen grains in one of the loculi. Fig. 5. Uninucleate tapetal cell. Fig. 6. Binucleate tapetal cell. Fig. 7. Fusion of two nuclei in tapetal cell. Fig. 8. Secondary uninucleate tapetal cell. Fig. 9. Four-nucleate tapetal cell. Fig. 10. Tapetal cell with a polyploid nucleus. Fig. 11. Microspore mother cell at diakinesis showing four bivalents and an extra body. Fig. 12. An isobilateral tetrad. Fig. 13. A linear tetrad. Fig. 14. A T-shaped tetrad. Fig. 15. A decussate tetrad. Fig. 16. A pentad. Fig. 17. A hexad. Fig. 18. An octad. Fig. 19. Nine microspores from one microspore mother cell.



FIGS. 20–28. Megasporogenesis of *Dipcadi serotinum*. Magnification ×575. Fig. 20. Nucellus with hypodermal archesporial cell. Fig. 21. Nucellus with archesporial cell and parietal cells. Fig. 22. Nucellus with two sporogenous cells. Fig. 23. Nucellus with enlarged megaspore mother cell. Fig. 24. Nucellus with megaspore mother cell at diakinesis showing four bivalents and an extra body. Fig. 25. Nucellus at first division of the megaspore mother cell. Fig. 26. Nucellus at diad formation. Fig. 27. Nucellus at diad formation with a third smaller nucleus organizing at the lower end of the spindle. Fig. 28. Nucellus at second division of megaspore mother cell with an early separation of chromosome in the upper diad cell.

present in the cell, might have led to the condition of polyspory. Pollen grains arising in this way may be nonfunctional as can be seen from Fig. 4, where some sterile pollen grains are seen in one of the loculi.

Megasporogenesis

The ovule is crassinucellate and a hypodermal cell in it differentiates as an archesporial cell (Fig. 20). The archesporial cuts off a parietal cell before functioning as a megaspore mother cell (Fig. 21). The parietal cell divides anticlinally and periclinally to form a two-layered parietal tissue (Figs. 23–25). The megaspore mother cell elongates considerably before it divides (Fig. 23). The spindle during the first division is straight and a diad is formed at the end of this division (Figs. 25, 26). Usually a linear tetrad is formed at the end of the second division (Fig. 31), but sometimes an obliquely linear tetrad is also formed (Fig. 33).

While the above-stated findings are normal features of megasporogenesis, many irregularities have also been observed. In Fig. 22, there are two cells which are differentiated from the rest of the cells of the nucellus. One is no doubt a megaspore mother cell as it is at typical synizetic stage. The cell below this has a conspicuous nucleus and dense cytoplasm indicating the possibility that it could function as another sporogenous cell.

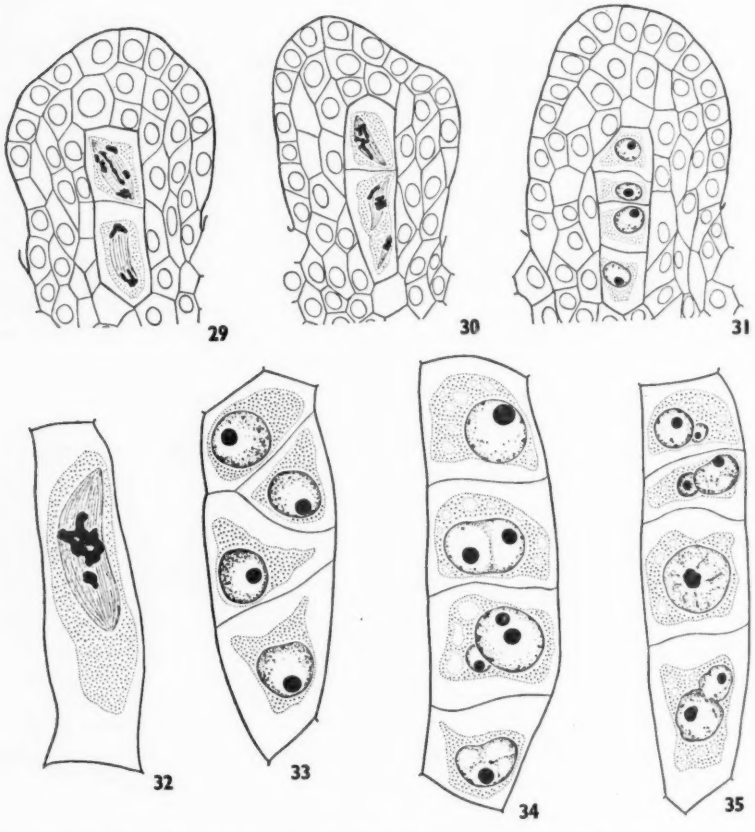
Figure 24 shows a diakinesis in which four bivalents are seen. The nuclear membrane is fairly distinct and intact. At the lower end of the same megaspore mother cell there is a heavily stained body which is probably chromosomal. It is rather interesting that such comparable stages could be observed during both microsporogenesis and megasporogenesis.

Figure 32, which is a metaphase of the first division of the megaspore mother cell, has a bivalent or so lying farther from the rest of the plate. At the end of the division, this may organize into a smaller nucleus in addition to the two normal nuclei of the diad. Probably a diad of the type as in Fig. 27 has resulted in this manner.

In the upper diad cell of Fig. 28 there is a chromosome lying separately at each end of the spindle while the rest of the chromosomes are at the equator of the spindle. During the second division, lagging or sticky nature of chromosomes is also noticed (Fig. 29). Another interesting case was observed wherein the lower diad cell had an additional spindle with a body of chromosomes (Fig. 30). If a third smaller nucleus had been formed during the diad stage as in Fig. 27, it would perhaps have divided to form such an extra spindle.

Figures 34 and 35 are of abnormal tetrads. In Fig. 34, the third megaspore from above has an extra, smaller nucleus. In Fig. 35, the upper two megaspores contain an extra nucleus; the nucleus of the third one is comparatively larger; that of the fourth megaspore is dumbbell-shaped. It has been on record that all the megaspores of a tetrad sometimes divide and form a binucleate condition as in *Gloriosa virescens* (1) and *Iphigenia indica* (10). In

these cases all the megaspores had developed to the two-nucleate stage of the embryo sac and both the nuclei of the binucleate megaspore were of equal size. However, in this plant of *D. serotinum*, the extra nuclei seen in the megaspores are smaller in size and they have been formed abnormally earlier during megasporogenesis. They should have been of the same size if the megaspore nucleus had divided to form a binucleate condition.



FIGS. 29–35. Megasporogenesis of *Dipcadi serotinum* (*continued*). Magnification: 29–31, ×575; 32–35, ×1130. Fig. 29. Nucellus at second division of megaspore mother cell with lagging or sticky chromosomes in the upper diad cell. Fig. 30. Nucellus at second division of megaspore mother cell with a third spindle and chromosomes at the lower end. Fig. 31. Nucellus with a linear tetrad of megaspores. Fig. 32. First division of megaspore mother cell with a bivalent or so lying separate from the rest. Fig. 33. An obliquely linear tetrad. Fig. 34. An abnormal tetrad with an extra, smaller nucleus in the third megaspore. Fig. 35. An abnormal tetrad with an extra, smaller nucleus in the upper two megaspores and a dumbbell-shaped nucleus in the lowermost megaspore.

Discussion

Dipcadi serotinum, being a Liliaceous member with low chromosome number, seems to be a fertile material for both cytological and embryological investigations. The occurrence of many irregularities in this plant is noteworthy and some of them are difficult to account for without a detailed study. All the chromosomes seemingly take part in the nucleolar organization; this and the occurrence of univalents are departures from the behavior of a normal diploid (cf. 11).

The occurrence of polyspory during microsporogenesis is another departure from the normal behavior. Polyspory is on record in some cases like *Fuchsia* (4), *Rosa* (14), in a variety of apple (17), in *Cuscuta reflexa* (9), in *Helixanthera ligustrina* (8), and in *Hyphaene indica* (13). Five to eight microspores instead of the normal four have been observed in an amphiploid of *Triticum–Agropyron* hybrids (2). Percival (15) has recorded the occurrence of binucleate microspores and polyspory in *Aegilops*–wheat hybrids. Polyspory is believed to be due to certain meiotic irregularities and laggards which separate late and organize into micronuclei. In *Cuscuta reflexa* it is caused by supernumarary divisions in one or more of the four microspores (9). In *Hyphaene indica* it is the result of subsequent division following meiosis (13). The manner by which they are formed in *D. serotinum* is not clear. However, such abnormalities definitely reduce the fertility of the plant.

To the best of our knowledge, the abnormal megaspore tetrads and the formation of a third spindle during the division of a diad described in this text have not been on record so far. It may be the extra body seen at diakinesis, the early or late separation of the chromosomes during anaphase, or the sticky nature of the chromsomes that is responsible for such tetrads with extra nuclei. Presumably most of the megaspores of such abnormal tetrads are nonfunctional. All these irregularities in this plant may be suggestive of its hybrid nature. Further study on this species would therefore be interesting.

Acknowledgments

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GROWTH AND METABOLISM OF ASPERGILLUS NIDULANS EIDAM IN SURFACE CULTURE[1]

KAISER NAGUIB

Abstract

Aspergillus nidulans was grown on glucose, fructose, sucrose, or maltose in a high-sugar medium favorable for fat formation. Glucose was more readily metabolized than the other sugars, giving a heavy felt with the highest fat content in a shorter time. Sucrose was inverted before absorption and was more conducive to growth than to fat formation. Maltose was probably absorbed after hydrolysis. It gave a heavy felt and a high fat yield but after a longer period of incubation than with glucose. Maltose metabolism was marked by lower nitrogen utilization. Hence, the maltose-fed mycelial felts had the highest carbohydrate content and the lowest protein content.

Growth proceeded as long as there was available sugar in the culture medium. Extracellular nitrogenous compounds appeared in the culture media before the complete exhaustion of the sugar in some cases, providing evidence that this process is not purely autolytic in nature. The early growth phase on the different sugars was characterized by low-fat, high-protein mycelial felts. Active fat formation was found to start after the drop of the nitrogen content of the medium to a very low level. The inverse relationship between fat and protein contents in the mycelium was traced during growth on glucose, sucrose, or maltose at different stages of growth. On fructose, except for the early growth period, fat and protein syntheses proceeded proportionally.

Introduction

The variation in behavior of different fungi towards different sugars when supplied in their culture media has been reported by several workers, e.g. Hawker (12), Hawker and Chaudhuri (14), Stockdale (37), v. Hofsten (18), Taber and Vining (39), and Townsend (40). This phenomenon does not seem to be confined to microorganisms but has been noted with higher plants as well (Robbins and Schmidt (31), Steinberg (36), Lee (21), Hildebrandt and Riker (16), etc.).

Dimond and Peltier (5) studied this phenomenon from an economical standpoint with the aim of promoting penicillin production by *Penicillium notatum*. Dealing with fat-forming microorganisms, Smedley-Maclean and Hoffert (35), Kaibara (19), and Garrido and Walker (10) found that the amount of fat produced differed with the type of sugar supplied in the culture medium.

It was thought that the study of this subject in more detail by going deeper into the metabolism of the fungus may throw some light on the observed variation in response of microorganisms to different sugars. In the present study the fat-forming fungus *Aspergillus nidulans* was grown on four different sugars under cultural conditions favorable for fat formation, and the synthesis of proteins, fat, and carbohydrates in the fungal mycelium was traced.

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Contribution from the Botany Department, Faculty of Science, University of Cairo, Egypt.

It was also noted that most of the conclusions arrived at were based on data obtained at a fixed time of harvest. As this does not necessarily give a real picture, as has been pointed out by Lilly and Barnett (22), estimations in the present work were carried out at different periods of incubation.

Materials and Methods

The Organism

A culture of *Aspergillus nidulans* Eidam brought from the Centraalbureau Schimmelculture, Baarn, Holland, was used. It was maintained on malt extract agar slopes and subcultured at intervals of 2 weeks.

Composition of Media, Inoculation, and Incubation

The basal medium was composed as follows (g/100 ml): Ammonium nitrate, 0.300; sodium dihydrogen phosphate, 0.730; potassium sulphate, 0.022; magnesium sulphate, 0.500; zinc sulphate, 0.005; and ferric chloride, 0.016. This basal medium proved to be favorable for fat formation by *Aspergillus nidulans* (Garrido and Walker (10)). The salts were first dissolved and the solution was adjusted with concentrated hydrochloric acid to pH 3.8. The solution was then divided into four equal portions and to each portion the appropriate sugar was added and the solution was then made up to volume with distilled water. The sugars used for the present study were glucose, fructose, sucrose, and maltose. They were added at the level of 17 g/100 ml medium. The media were then dispensed at 25 ml portions into 100-ml conical flasks. The flasks were then plugged and sterilized at 10 lb for 15 minutes. The cultures were inoculated with a spore suspension made from 2-week-old slant cultures on malt agar. The culture flasks were incubated at 25° C. Quintuplicate sets were withdrawn at intervals of 4, 6, 8, 10, 12, 14, and 17 days for estimation of total nitrogen and residual sugar in the media, and total nitrogen, protein nitrogen, fat, and total carbohydrate in the mycelial felts.

Methods of Analysis

Estimation of reducing sugars in the media was carried out by a modified Schaffer-Hartmann's method as given by Saïd and Naguib (34) in their work on *Fusarium moniliforme*. This gives an estimate of the residual sugar in the glucose and fructose media. In the sucrose and maltose media, and for the total carbohydrate content in the mycelial felts, estimation was carried out after acid hydrolysis.

Estimation of total nitrogen in the culture media and of total nitrogen and protein nitrogen in the mycelial felts was performed by a micro-Kjeldahl method as described by Naguib and Walker (26).

Estimation of fat in the mycelial felt was carried out by the method of Woodbine, Gregory, and Walker (42).

Results

Sugar Analysis in the Culture Media and Sugar Uptake

From Fig. 1, it can easily be seen that the biggest drop in sugar concentration in the early period of growth was in the maltose medium; glucose and sucrose media were not appreciably different, but the fructose medium showed the smallest drop. As time went on, the glucose medium showed a steep drop in its sugar concentration exceeding that in the maltose medium and was completely depleted in 10 days. During the later period of growth, the sucrose medium showed a similar trend to that of the fructose medium. Maltose, sucrose, and fructose media were all depleted by the 14th day.

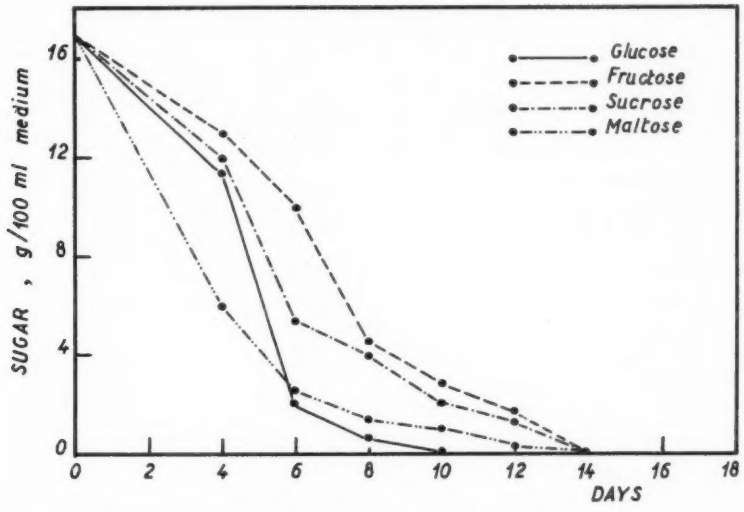


FIG. 1. Changes in the total sugar content of the different media with time (expressed as g glucose).

It was also found that, in the sucrose media, reducing sugars appeared and accumulated until on the eighth day from inoculation the sugar content of these media was almost completely made up of reducing sugars. Appearance of reducing sugars in the sucrose cultures showed that sucrose has been inverted into glucose and fructose and that the inversion products were partly absorbed and partly left in the external media. Moreover, it seemed that after inversion, glucose was absorbed preferentially to fructose from the culture medium; the drift in the sugar concentration in the sucrose medium approached the glucose medium in the early growth period and the fructose medium in the later period when fructose predominated. Sucrose inversion and the preferential absorption of glucose from the inversion products in fungal cultures has been previously reported by Dimond and Peltier (5), Saïd and Naguib (33), and Mandels (24). Forsyth and Webley (9) observed

this in bacterial cultures, and Harley and Smith (11) in cultures of *Peltigera polydactyla* disks. It has also been recorded in higher plant tissues by Saïd (32), Burström (3), and Street and Lowe (38).

The disappearance of maltose from the culture medium proceeded at a higher rate than glucose during the early growth and than fructose and sucrose throughout the experimental period. It may be assumed then that maltose was also hydrolyzed in the culture medium into glucose which, being nascent and active, was more easily taken up than ordinary glucose. It may also be suggested that the energy released during hydrolysis might enhance sugar utilization. Conclusions arrived at concerning maltose absorption are still controversial (cf. Saïd (32), Saïd and Naguib (33), Burström (3), etc.).

Nitrogen Absorption and Accumulation in the Mycelial Felts

Disappearance of nitrogen from the different cultures (Fig. 2) proceeded steadily down to a minimum value after which the nitrogen content started to rise again. The least amount of nitrogen that remained in the culture medium, and the biggest amount that diffused back, were in the glucose-fed cultures. The maltose cultures, on the other hand, left in the medium a relatively big amount of nitrogen unutilized. Moreover, the nitrogen content of these cultures started to rise earlier than in the other sugar media.

The drift in the nitrogen content in the culture media was reflected in the nitrogen content of the mycelial felts. The appearance of extracellular

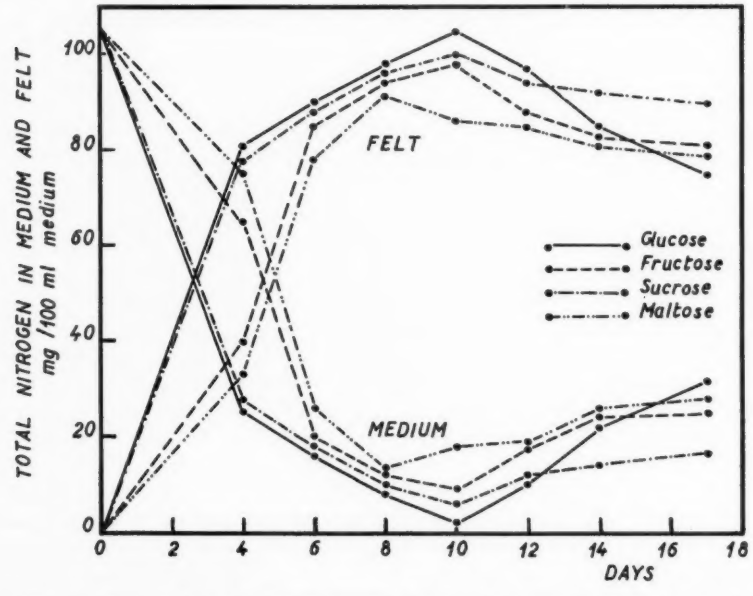


FIG. 2. Changes in total nitrogen content of the different media and of the mycelial felts with time.

nitrogen in the culture medium was concurrent with a drop in the mycelial nitrogen. This release of nitrogenous compounds into the external medium has been recorded by several workers, e.g. Hockenhull (17), Pai (27), Morton and Broadbent (25), Fogg (7), v. Hofsten (18), and Naguib and Walker (26), though the physiological significance of this phenomenon is not yet clearly understood. Pai (27) considered the increase in residual nitrogen in cultures of *Fusarium moniliforme* and *Fusarium vasinfectum* to be an autolytic process since it occurred after complete exhaustion of the sugar in the external media. Morton and Broadbent (25), who dealt with the problem in more detail, concluded that the extracellular nitrogenous material is not simply a product of autolysis or senescence. Fogg (8) has also pointed out that, in *Anabaena cylindrica*, the total amount of extracellular substances is relatively greatest in young cultures and so they are evidently not produced by autolysis of dead cells. The results obtained in the present study show that the residual nitrogen increased after exhaustion of the sugar in the case of glucose cultures only. On the other sugars, however, rise in the nitrogen content of the culture medium occurred before the complete depletion of its sugar content.

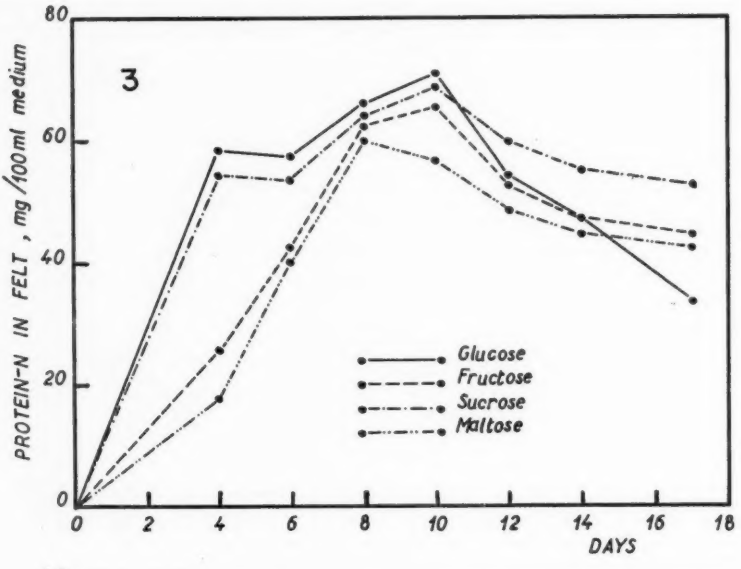
Protein Content of the Mycelial Felts

As can easily be noted from Fig. 3, protein synthesis in mycelial felts fed with glucose or sucrose continued at a markedly high rate in the first 4 days after which it subsided for the following 2 days. Then it increased again until it attained its maximum on the tenth day. In felts fed with fructose or maltose the protein content increased steadily but at a lower rate than with glucose or sucrose up to a maximum on the tenth day on fructose and on the eighth day on maltose. After reaching the maximum, the protein content dropped steadily in felts fed with glucose and slightly on those fed with the other sugars until the expiry of the experimental period.

Fat Content of the Mycelial Felts

Fat synthesis in the mycelium on all the experimental sugars (Fig. 4) proceeded at a markedly low rate during the early stages of growth. It became active and steady by the sixth day from inoculation in felts of the maltose cultures, and by the fourth day in the other felts. In this respect, fat formation seems to be quite different from protein synthesis which started in all the mycelial felts right from the beginning. Glucose, within the experimental conditions, proved to be the sugar most conducive to fat formation by *Aspergillus nidulans*. It gave the maximum fat yield, amounting to about 46% of the felt dry weight in 8 days. The highest fat yield next to that of glucose cultures was obtained on maltose in 2 weeks. By this time, the glucose-fed felts contained the least amount of fat.

The fat contents of felts grown on fructose or sucrose were intermediate between those fed with glucose and those with maltose; but while sucrose led to higher fat content than fructose in the young felts, both sugars were almost equally conducive to fat formation in the old felts.



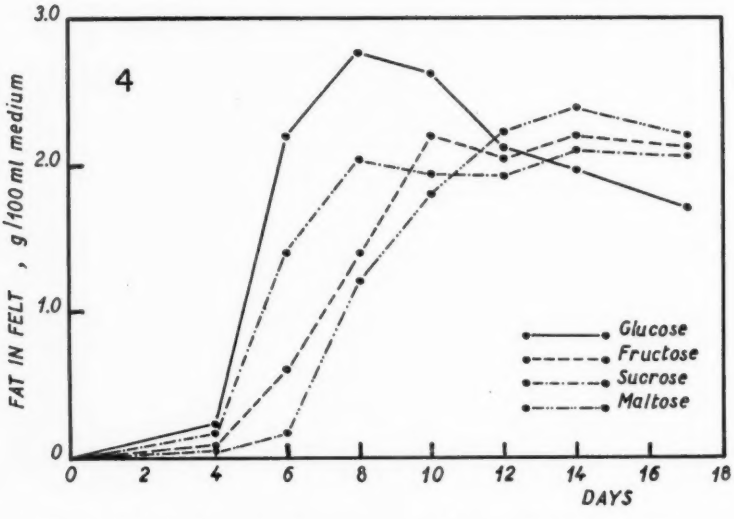


FIG. 3. Changes in protein nitrogen content of the mycelial felts with time.
FIG. 4. Changes in fat content of the mycelial felts with time.

Concerning fat formation by other microorganisms from different sugars, it may be mentioned that Lockwood and co-workers (23) working with *Penicillium javanicum* found that sucrose and maltose were inferior to glucose in this respect. Reichel and Reinmuth (30), on the other hand, found that fructose and sucrose were more easily converted to fat than glucose in *Endomyces vernalis*. Kleinzeller (20) reported that *Torulopsis lipofera* could use glucose, fructose, and sucrose equally well for fat synthesis.

Total Carbohydrate Content of the Mycelial Felts

As can be noted from Fig. 5, the total carbohydrate content increased gradually and steadily as growth went on until a maximum was reached by the 10th–12th days. Then it declined until the end of the experimental period. During the logarithmic growth period the mycelial felts grown on maltose had relatively low carbohydrate content. In older felts, maltose induced the maximum carbohydrate synthesis, while glucose induced the minimum.

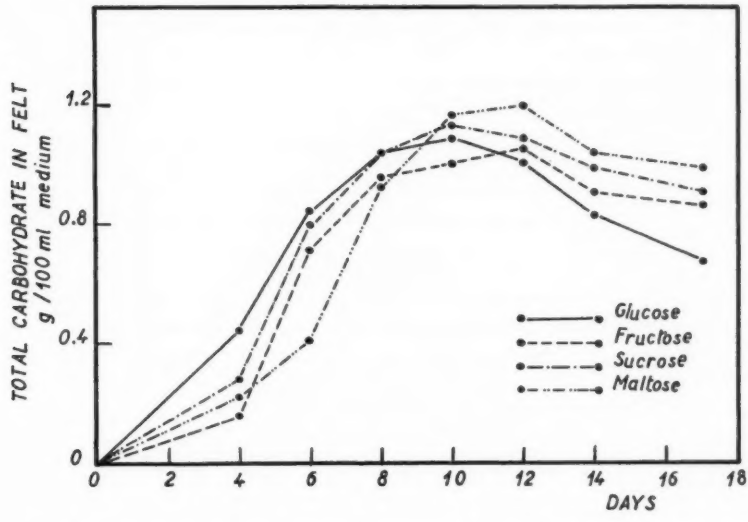


FIG. 5. Changes in total carbohydrate content of the mycelial felts with time (expressed as g glucose).

Dry Weights of the Mycelial Felts

From Fig. 6, it can be seen that growth, as measured by the dry weight increase of the mycelial felts, appeared to be more rapid on glucose than on the other sugars so that maximum dry weight occurred on the eighth day on glucose and on the 14th day on other sugars. The biggest amount of felt was obtained on sucrose but was not much different from that obtained on

glucose or maltose. It is also interesting to notice, from comparing Figs. 1 and 6, that growth continued so long as there was available sugar in the culture media; when the media were depleted, dry weight started to decline.

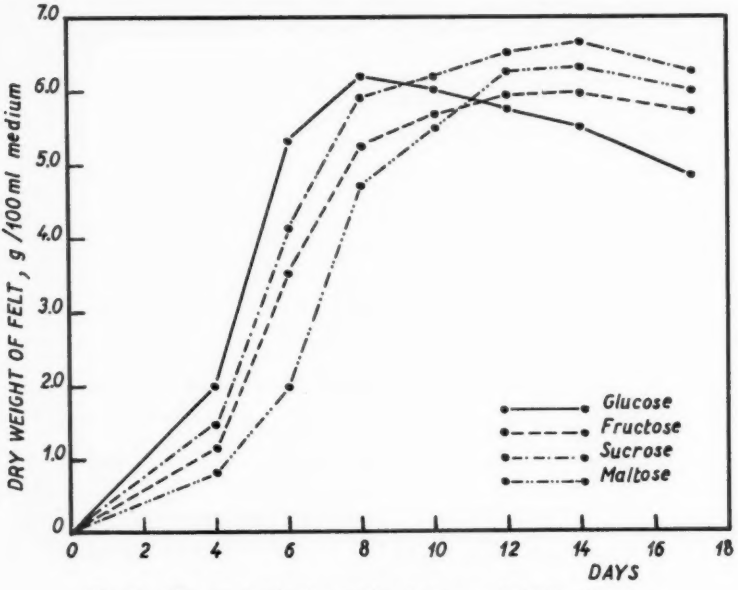


FIG. 6. Changes in the dry weight of the mycelial felts with time.

Discussion

The results presented in Figs. 1 and 6 of the present investigation show that *Aspergillus nidulans* could utilize glucose more readily than the other sugars used. The glucose media were completely depleted of their sugar content in a comparatively shorter period with a higher rate of growth leading to a relatively high yield of felt. It could also be noted that autolysis, as indicated by drop in the dry weight of the mycelial felts, has started on glucose rather earlier and more rapidly than on the other sugars. From Fig. 6, it can be seen that nitrogenous compounds have escaped from the mycelial cells into the external media. In the glucose cultures, this escape of nitrogenous compounds occurred during the decline in the dry weight of the mycelial felts and after the exhaustion of the sugar of the culture medium. In the other cultures, however, it took place before the complete disappearance of the sugar from the culture media and before the decline of the dry weights of the mycelial felts. It is suggested, therefore, that this process is not purely of autolytic nature.

In the maltose cultures, the higher rate at which maltose was disappearing from the culture medium during the early growth period as indicated above did not correspond to a higher rate of building up of mycelial felt. There

was, on the contrary, a relatively smaller increase in the amount of mycelium produced. It may be assumed therefore either that maltose has been consumed in the process of respiration to a greater extent, or that it disappeared as sugar but has been transformed into other intermediate compounds that accumulated in the external media and were later utilized in growth. With respect to the former assumption, it may be mentioned that Hawker (13) found that *Melanospora destruens* respired on maltose at a higher rate than on glucose, fructose, or sucrose.

In view of the results presented in Fig. 2, it is interesting to point out that in spite of the relatively higher rate of maltose utilization shown above, a relatively lower nitrogen supply was required throughout the incubation period. This eventually resulted in a lower yield of mycelial felt in the earlier stages of growth, a greater part of the sugar being utilized in noncellular products. Protein content, however, declined long before dry weight was maximum so that, in the later period of incubation, the relatively good yield of mycelial growth produced contained higher amounts of carbohydrates and fat but lower amounts of proteins. Investigations carried out in the same laboratory by Garrido and Walker (10) have shown that the ratio of fat to substances other than fat in the felt produced on maltose was much less than in the felt grown on glucose. This may therefore be attributed, in the light of the present findings, to the higher amount of total carbohydrate formed on feeding with maltose (Fig. 5). It would be of interest to recall here that Smedley-Maclean and Hoffert (35) found that incubation of yeast cells with maltose solutions gave rise to a large increase in total carbohydrate content and to a decrease in the fat content in comparison with results obtained when solutions of glucose, fructose, or sucrose were used.

In sucrose cultures, higher amounts of nitrogen have been utilized than in maltose cultures, and therefore the mycelial felts produced have later accumulated higher amounts of proteins but lower amounts of fat and of total carbohydrate.

Moreover, tracing the growth of *Aspergillus nidulans* on the various sugars under conditions favorable for fat formation as originally planned has revealed that during the early growth period, increase in the dry weight of cellular material was mainly due to the building up of complicated nitrogenous compounds and to accumulation of carbohydrates rather than to fat formation. On maltose, fat formation was even more delayed than on the other sugars. On sucrose and glucose, protein synthesis seemed to have almost ceased from the fourth to the sixth day of incubation. Simultaneously, however, there was a sudden rise in the fat content of mycelial felts grown on these sugars, concurrent with a big drop in the sugar concentration in the culture media. The tendency during this growth phase was towards the accumulation of fat and carbohydrates rather than proteins. On fructose and maltose, the process of fat synthesis did not jump to the same extent as on the two other sugars. This is probably due to the fact that the process of building up of complicated nitrogenous compounds necessary for protoplasmic synthesis was proceeding rather actively.

The fact that active fat formation started in mycelial felts fed with glucose or sucrose earlier than in those fed with maltose as mentioned above may be correlated with the nitrogen content of the medium at that time. It seems that only when a low nitrogen level is reached does fat formation occur actively. The required low nitrogen level was attained in 4 days in the glucose or sucrose medium, but in 6 days in the maltose medium as can be seen from Figs. 2 and 4. This conclusion has its parallel in that arrived at by Collyer and Fogg (4) in their studies in *Chlorella pyrenoidosa*. They concluded that primary limitation for fat synthesis is imposed by the concentration of available nitrogen in the medium and only when this falls below a certain level is fat accumulation considerable. In this connection, it is worth while to refer to the work of Witter and Stotz (41). These investigators could separate processes of growth from those of fat formation by *Fusarium lycopersici* by growing the mold on full nutrient medium replaced after growth by nitrogen-free, high-sugar medium.

The present findings seem also to throw some light on the protein–fat relationship. Studies on a variety of microorganisms by several workers, e.g. Raaf (29), Enebo and co-workers (6), Bernhauer and Rauch (1), and Naguib and Walker (26), have shown that there is an inverse relationship between protein and fat syntheses, the most important governing factor being the C:N ratio. With initially high C:N ratio, microorganisms are put under circumstances favorable for fat formation, as has been shown by Prill and co-workers (28), Heide (15), Bichkovskaya (2), and Kleinzeller (20). In the present study, the culture media with high C:N ratio were used, with the intention of following as well the protein–fat relationship during the growth of *Aspergillus nidulans* on the different sugars used. During early growth on glucose or sucrose, protein synthesis was proceeding very actively, while fat synthesis had barely started. This was followed by another phase when protein synthesis declined while fat synthesis jumped to a comparatively high rate. In the maltose cultures, this inverse relationship was not so clearly manifested until on the eighth day when mycelial protein started to decrease while the fat content continued to increase until a maximum was reached on the 14th day. On fructose, however, the inverse relationship between fat and protein contents was only shown during early growth. Otherwise, the protein synthesis seemed to go hand in hand with fat synthesis. It is interesting to mention in this connection that Collyer and Fogg (4) came across this inverse relationship in some algae, while in other algae there was the tendency for the amounts of proteins and fat to be directly proportional. It seems therefore that the inverse relationship between proteins and fat varies also with the different nutritive conditions and during the progress of growth.

Acknowledgment

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CYTOGENETICS OF SOLID STEM IN COMMON WHEAT

II. STEM SOLIDNESS OF MONOSOMIC LINES OF THE VARIETY S-615[1]

RUBY I. LARSON AND M. D. MACDONALD[2]

Abstract

Lines of a variety of common wheat, S-615, monosomic for chromosomes III and XVI of homoeologous group 3 had culms less solid in the top internode than normal S-615. Monosomics of homoeologous group 5, namely, V, IX, and XVIII, were less solid in the bottom four internodes than S-615. These five chromosomes carry genes for solid stem in this variety. Monosomics XIX, XX, and XXI, the D-genome chromosomes of homoeologous groups 6, 2, and 7 respectively, were more solid than the normal check in both top and lower internodes, indicating that the missing chromosomes carry genes for hollow stem. Chromosome XIII, a homoeologue of XX, which in Chinese Spring has a gene for hollow stem, does not affect the amount of pith in the culm of S-615.

The concept of the culm phenotype in a given environment resulting from an interaction of genes promoting pith development and those opposing it makes it possible to reconcile results of genetic experiments on solid stem in wheat that previously appeared contradictory.

Introduction

Varieties of wheat in which most of the culm is filled with pith are referred to as solid-stemmed wheats. The two varieties of common wheat, *Triticum aestivum* L. *emend* Thell., licensed in Canada as resistant to the wheat stem sawfly, *Cephus cinctus* Nort., are Rescue and Chinook, both of which are solid-stemmed. Since both derive their solidness from the variety S-615, the genetics of solid stem in S-615 is of special interest.

Platt *et al.* (9) showed that S-615 differs from two hollow-stemmed varieties of common wheat by three recessive genes for solid stem. Analysis of pentaploid hybrids revealed that the D genome is largely responsible for the thin-walled hollow culms of most hexaploid wheats, but genes of the A and B genomes contribute to it. Literature on the genetics of solid stem in wheat was reviewed in a previous paper of this series (4).

Tests of solid stem in F_2 hybrids of all 21 monosomics of Chinese Spring by S-615 showed that these two varieties differ by at least four genes affecting pith content of the culm (4). Chinese Spring has a gene for hollow stem on chromosome XIII that S-615 does not have. Lines monosomic for chromosomes II and XX were more solid than the normal showing that in Chinese Spring these homoeologues of XIII tend to produce a hollow culm, but ratios could not be interpreted on a factorial basis. Monosomics XIX and XXI were more solid than normal but ratios were anomalous. This suggested that these chromosomes carried genes for hollow stem in both varieties. Chromosome VIII of Chinese Spring was shown to have a gene for solid stem not found in S-615.

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As no chromosomes in S-615 were identified as carrying genes for solid stem it was concluded that such genes were probably recessive, as Platt *et al.* (9) showed, and hence could not be detected by the monosomic F_2 method when Chinese Spring was the monosomic parent. They could, however, be readily detected in monosomic lines of S-615, as the loss of one member of a pair of critical chromosomes would eliminate most of the effect of the gene carried thereon. The purpose of these studies was to determine the effect of the loss of each chromosome on the amount and distribution of pith in S-615.

Materials and Methods

Monosomic Lines of S-615

Monosomic lines of S-615 were produced by crossing Sears' monosomic lines of Chinese Spring by S-615 and backcrossing by S-615 for seven generations using monosomics as female parents in each generation. At the end of the backcross program monosomic plants from the final backcross were allowed to self, and seed from both monosomic progeny and normal progeny was saved. The derived normals were used as checks because in spite of seven backcrosses by S-615 the lines might still retain some Chinese Spring germ plasm, and without these checks it would be impossible to tell whether any deviation from S-615 was due to the loss of a chromosome or to the genetic constitution of the line. Although usually about a quarter of the progeny of a selfed monosomic line are normal (11), in some monosomic lines of S-615 normal plants are not that frequent. Because of insufficient seed, normal plants from S-615 lines monosomic for chromosomes I, V, XIV, XV, and XVIII were not grown. The check for monosomic VII was mainly nullisomic VII. Lines involving chromosomes XIII and XVIII had only six backcrosses and those involving chromosome XIV had only five.

Design

Lines of S-615 monosomic for each of the 21 chromosomes were grown on irrigated land at Lethbridge in 1954 to test them for solid stem. Normal S-615 from regular stock and derived normals of all monosomic lines except I, V, XIV, XV, and XVIII were grown as checks. All but one of the four replicates of supposed normal lines from mono-VII proved to be nullisomic, so mono-VII was tested mainly against nulli-VII. Henceforth monosomic lines of S-615 will be designated by S and the number corresponding to the missing chromosome, e.g., SI, SII, and the corresponding derived normals will be called SI normal, SII normal, etc. Monosomic lines of Chinese Spring, and their derived normals originally included in the test, were damaged too badly by hail to give reliable data. Additional solid-stemmed check varieties were Rescue, Chinook, and Golden Ball (*T. durum* Desf.).

The original planting was a 9×9 lattice with four replicates. Each plot was a single 10 ft row in which 50 seeds were sown.

Examination for Solid Stem

All plants were pulled for examination in the laboratory except those occupying about six inches at each end of the plot. The longest culm from each of 10 plants of each plot was examined. In those lines in which it was possible to distinguish monosomic plants from normals, namely, SII, SIV, SV, SVIII, SIX, SXIII, SXVI, SXVIII, and SXX, only monosomics were examined. Since on the average a quarter of the plants in lines in which the monosomics were not morphologically distinct were probably normal, the effect of the loss of the chromosome in such lines was underestimated.

Solid stem indices were determined by a modification of the method used in the analysis of the F_2 of Chinese Spring monosomics by S-615 (4). The method, which was used routinely in solid stem ratings at this laboratory, is here referred to as the standard method, and the seven locations of cuts in the culm as standard cuts. Although these cuts have been satisfactory for studies of segregating populations in which few plants had very solid culms, they are not adequate for comparison of lines in which most plants may be expected to be solid in the centers of internodes II, III, IV, and V. Therefore, special cuts were made near the top of each of these internodes at which points the culms usually have at least a small cavity. This part of the internode is very important in resistance to the wheat stem sawfly, *Cephus cinctus* Nort., because most eggs appear to be laid here. A cavity here favors hatching and survival of first-instar larvae. Cuts were located 2 cm from the top in internodes II and III, and 1 cm from the top of internodes IV and V. Any internode 3 cm or less in length was cut only at the center, and its rating was taken as the value for both standard and special cuts. In culms having only four internodes, solid stem ratings for internode IV were used as estimates for V.

Analysis of Solid Stem Data

Standard total solid stem indices were calculated by adding the indices for the top internode to those for the center cuts of the bottom internodes; special total solid stem indices were calculated by adding the indices for the top internode to those of the special cuts in the lower internodes. Analysis of variance was performed on the two sets of data by the method for randomized blocks rather than for lattice, owing to loss of many lines, and least significant differences were calculated. Graphs were made of solid stem gradients of each monosomic line and its check (derived normal for all but SI, SV, SXIV, SXV, and SXVIII, for which S-615 was used), and of Rescue, Chinook, and Golden Ball with S-615 as check. As the graphs revealed striking differences in pith distribution, analysis of variance was performed separately on each individual point of cut.

Results

The total solid stem indices for standard and special cuts of all lines are summarized in Table I. Monosomic lines are placed in their homoeologous groups (11). Where known, the genome to which it belongs is placed in

parenthesis after the chromosome number. Brackets around data for SVII "normal" indicate that the line was mainly nullisomic.

None of the derived normals was significantly different from S-615 according to the standard cuts, but those derived from monosomics SIX and SXI were more solid than S-615 according to the special cuts. SIII and SXVI of the third homoeologous group, and SV and SIX of the fifth homoeologous group, were less solid than their checks (derived normals when present, otherwise S-615) by both ratings. SXX of the second homoeologous group, and SXXI of the seventh homoeologous group, were more solid than their normal checks according to both ratings; SXI of the latter group was more solid than S-615 but not more so than its derived normal, which was unusually solid. The

TABLE I

Total solid stem indices in S-615 monosomic lines, their derived normal lines, and check varieties, Lethbridge, 1954

Homoeologous group	Line	Genome	Standard cut		Special cut	
			Monosomic	Normal	Monosomic	Normal
1	SI	(B)	29.42	—	26.32	—
	SXIV	(A)	31.30	—	28.65	—
	SXVII	(D)	30.68	30.55	27.50	27.45
2	SII		29.78	30.38	25.85	27.05
	SXIII		30.80	30.50	27.82	26.40
	SXX	(D)	32.20††	29.58	30.45††	26.02
3	SIII	(B)	25.05**	30.90	22.05**	27.88
	SXII	(A)	31.72	31.18	29.45	28.30
	SXVI	(D)	28.32**	30.78	25.05*	27.82
4	SIV	(A)	32.28	30.80	30.48†	27.60
	SVIII	(B)	30.98	30.00	28.72†	26.35
	SXV	(D)	30.72	—	28.02	—
5	SV	(B)	26.65**	—	23.95*	—
	SIX	(A)	28.42**	31.30	26.12**	29.40†
	SXVIII	(D)	29.78	—	27.15	—
6	SVI	(A)	31.55†	29.92	29.10	26.88
	SX	(B)	30.42	29.60	28.28†	25.55
	SXIX	(D)	31.50	29.92	30.05††	26.58
7	SVII	(B)	30.60	(30.92)	27.50	(28.00)
	SXI	(A)	32.22	31.72	29.05	29.78††
	SXXI	(D)	33.32††	30.50	32.90††	26.78
	S-615		—	30.15	—	26.48
	Rescue		—	28.80	—	27.68
	Chinook		—	29.72	—	28.28
	Golden Ball		—	32.85††	—	32.52††
	L.S.D. .05			1.60		2.34
	.01			2.12		3.09

*Less solid than check.
†More solid than check.
NOTE: The monosomics are compared with the corresponding normals when these are present and with S-615 when they are not; normals are compared with S-615.

A- and B-genome monosomics SIV and SVIII in the fourth homoeologous group were more solid than their checks according to both ratings. In the sixth homoeologous group, SVI, standard, and SX and SXIX, special, were more solid than their checks.

Presumably, when a monosomic is less solid than normal its critical chromosome promotes pith development and when a monosomic is more solid than normal its critical chromosome inhibits pith development. It is shown in Table I that the D-genome chromosomes XIX, XX, and XXI have the most potent inhibitors in their respective homoeologous series. Chromosomes of the A and B genomes are less effective. On the other hand, three of the four chromosomes needed to produce a total solid stem index equivalent to that of the normal checks, III, V, and IX, are in the A and B genomes, only XVI being in D.

TABLE II

Solid stem indices of three separate cuts in the top internode of S-615 monosomic lines, their derived normal lines, and check varieties, Lethbridge, 1954

	Cut					
	1		2		3	
Line	Mono	Normal	Mono	Normal	Mono	Normal
SI	3.60	—	4.08	—	3.88	—
SXIV	3.40	—	4.10	—	4.40††	—
SXVII	3.75	3.55	4.10	4.15	3.78	3.62
SII	3.50	3.55	4.08	4.00	3.75	3.75
SXIII	3.45	3.62	4.10	4.12	3.98	3.70
SXX	3.92††	3.40	4.75†	4.28†	4.42††	3.68
SIII	3.05**	3.55	2.82**	4.20	1.82**	4.05
SXII	3.68	3.62	4.30	4.28†	4.25	3.82
SXVI	3.58	3.72	3.65**	4.25†	2.40**	3.98
SIV	3.85	3.72	4.30	4.22	4.52†	3.90
SVIII	3.80	3.68	4.32	4.10	4.08†	3.50
SXV	3.58	—	4.20	—	4.18†	—
SV	3.25	—	3.62	—	3.32	—
SIX	3.12**	3.85†	3.82**	4.32†	4.20	3.78
SXVIII	3.75	—	4.30†	—	4.18†	—
SVI	3.75	3.38	4.35†	3.90	4.05	3.62
SX	3.70†	3.22	4.22	3.98	4.08	4.08
SXIX	3.88†	3.50	4.28	3.92	3.85	3.55
SVII	3.38	(3.32)	4.20	(4.10)	4.05	(4.18)†
SXI	3.60	3.85†	4.45	4.10	4.55	4.12
SXXI	4.38††	3.50	4.70††	4.20	4.38†	3.78
S-615	—	3.45	—	3.85	—	3.62
Rescue	—	4.08††	—	3.50	—	1.90**
Chinook	—	4.32††	—	4.82††	—	1.92**
Golden Ball	—	4.60††	—	4.85††	—	4.95††
L.S.D. .05	.38		.38		.52	
.01	.50		.50		.69	

*Less solid than check.
†More solid than check.

Data on individual cuts are summarized for the top internode in Table II, for standard cuts of the lower internodes in Table III, and for special cuts in the lower internodes in Table IV. The solid stem gradients are illustrated by graphs in Fig. 1; the points for solid stem index of special cuts are placed on the graph opposite the same cut number on the abscissa as the points for standard cuts to facilitate comparisons. The points representing the solid stem indices of special cuts are joined by thinner lines than those joining the other points.

The following general observations are evident:

(1) Standard cuts in the lower internodes are more effective in identifying lines less solid than normal, and special cuts are more effective in identifying lines more solid than normal, e.g., homoeologous group 5 vs. SXXI.

TABLE III

Solid stem indices of separate cuts in S-615 monosomics, their derived normal lines, and check varieties, Lethbridge, 1954. Standard cuts (center), bottom internodes

	Internode							
	II		III		IV		V	
Line	Mono	Normal	Mono	Normal	Mono	Normal	Mono	Normal
SI	3.98**	—	4.65	—	4.62	—	4.65	—
SXIV	4.75	—	4.98	—	4.90	—	4.78	—
SXVII	4.55	4.70	4.78	4.78	4.92	4.85	4.80	4.90
SII	4.52	4.65	4.70	4.88	4.60	4.82	4.62	4.72
SXIII	4.72	4.55	4.88	4.88	4.82	4.82	4.85	4.80
SXX	4.58	4.42	4.98†	4.68	4.82	4.60	4.72	4.52**
SIII	3.30**	4.62	4.58*	4.92	4.68	4.80	4.80	4.75
SXII	4.75	4.68	4.98	4.90	4.95	4.92	4.82	4.95
SXVI	4.18	4.18*	4.90	4.95	4.85	4.88	4.78	4.82
SIV	4.82	4.55	4.90	4.80	4.95	4.75	4.92	4.82
SVIII	4.62	4.68	4.80	4.72	4.72	4.70	4.62	4.62*
SXV	4.32	—	4.85	—	4.88	—	4.72	—
SV	3.82**	—	4.22**	—	4.12**	—	4.28**	—
SIX	4.00**	4.70	4.32**	4.92	4.40**	4.88	4.55*	4.85
SXVIII	4.15*	—	4.60	—	4.42**	—	4.38**	—
SVI	4.80	4.62	4.90	4.75	4.88	4.82	4.82	4.82
SX	4.48	4.22*	4.70	4.78	4.62	4.68	4.62	4.65
SXIX	4.75	4.65	5.00	4.80	4.88	4.75	4.88	4.75
SVII	4.75	(4.72)	4.78	(4.85)	4.75	(4.90)	4.70	(4.85)
SXI	4.92	4.80	4.92	4.92	4.92	4.95	4.85	4.98
SXXI	4.92	4.55	5.00	4.98	4.98	4.78	4.98†	4.72
S-615	—	4.68	—	4.85	—	4.82	—	4.88
Rescue	—	4.52	—	5.00	—	5.00	—	4.80
Chinook	—	4.78	—	4.75	—	4.62	—	4.50**
Golden Ball	—	4.90	—	4.92	—	4.32**	—	4.30**
L.S.D. .05		.43		.29		.25		.25
.01		.56		.38		.33		.32

*Less solid than check.
†More solid than check.

(2) D-genome monosomics (on the right of Fig. 1) are usually more solid than other members of a homoeologous group that has solid-stemmed members, e.g., XX of group 2; group 4 is an exception.

(3) Within a group the solid stem gradients of members that are affected by loss of the chromosome deviate from normal in the same direction, e.g., SIII and SXVI in group 3.

By homoeologous groups the results are as follows:

In the homoeologous group 1 only SXVII had a derived normal as a check, and the monosomic was not significantly different from it in solid stem index at any point. Figure 1 shows that SI was less solid than S-615 in the standard cut of internode II and SXIV was more solid than S-615 at cut 3 of internode

TABLE IV

Solid stem indices of separate cuts in S-615 monosomics, their derived normal lines, and check varieties, Lethbridge, 1954. Special cuts (below top node), bottom internodes

	Internode							
	II		III		IV		V	
Line	Mono	Normal	Mono	Normal	Mono	Normal	Mono	Normal
SI	2.98	—	3.42	—	4.05	—	4.32	—
SXIV	3.68	—	3.98	—	4.48	—	4.62	—
SXVII	3.05	3.40	3.62	3.80	4.52	4.32	4.68	4.60
SII	2.95	3.38	3.35	3.85	4.00	4.25	4.22	4.28
SXIII	3.75	3.15	3.72	3.38	4.22	4.02	4.60	4.40
SXX	3.90	3.30	4.15††	3.25	4.65††	3.78*	4.65	4.35
SIII	2.12**	3.48	3.35	3.68	4.30	4.28	4.58	4.55
SXII	3.85	3.38	4.32	3.85	4.48	4.50	4.58	4.85
SXVI	2.78	3.35	3.85	3.72	4.30	4.22	4.50	4.58
SIV	4.20†	3.55	4.12†	3.45	4.62	4.22	4.85†	4.52
SVIII	4.20††	3.20	3.68	3.35	4.22	4.12	4.42	4.40
SXV	3.55	—	3.90	—	4.18	—	4.45	—
SV	2.88	—	3.15	—	3.70*	—	4.02**	—
SIX	3.18**	4.00††	3.50*	4.10	3.75**	4.60	4.55	4.75
SXVIII	3.40	—	3.48	—	3.90	—	4.15**	—
SVI	3.72	3.32	4.02	3.68	4.52	4.40	4.68	4.58
SX	3.55††	2.72	3.85†	3.10	4.42	4.05	4.45	4.40
SXIX	4.48††	3.18	4.40†	3.70	4.55	4.22	4.62	4.50
SVII	3.30	(3.38)	3.55	(3.92)	4.40	(4.50)	4.62	(4.60)
SXI	3.30*	3.92†	3.78	4.30†	4.60	4.70	4.75	4.78
SXXI	4.75††	3.28	4.82††	3.58	4.90††	4.05	4.98††	4.40
S-615	—	3.12	—	3.55	—	4.28	—	4.60
Rescue	—	4.05††	—	4.70††	—	4.72	—	4.72
Chinook	—	4.12††	—	4.10	—	4.45	—	4.52
Golden Ball	—	5.00††	—	4.80††	—	4.05	—	4.28*
L.S.D. .05	.61		.60		.46		.31	
.01	.81		.80		.61		.41	

*Less solid than check.
†More solid than check.

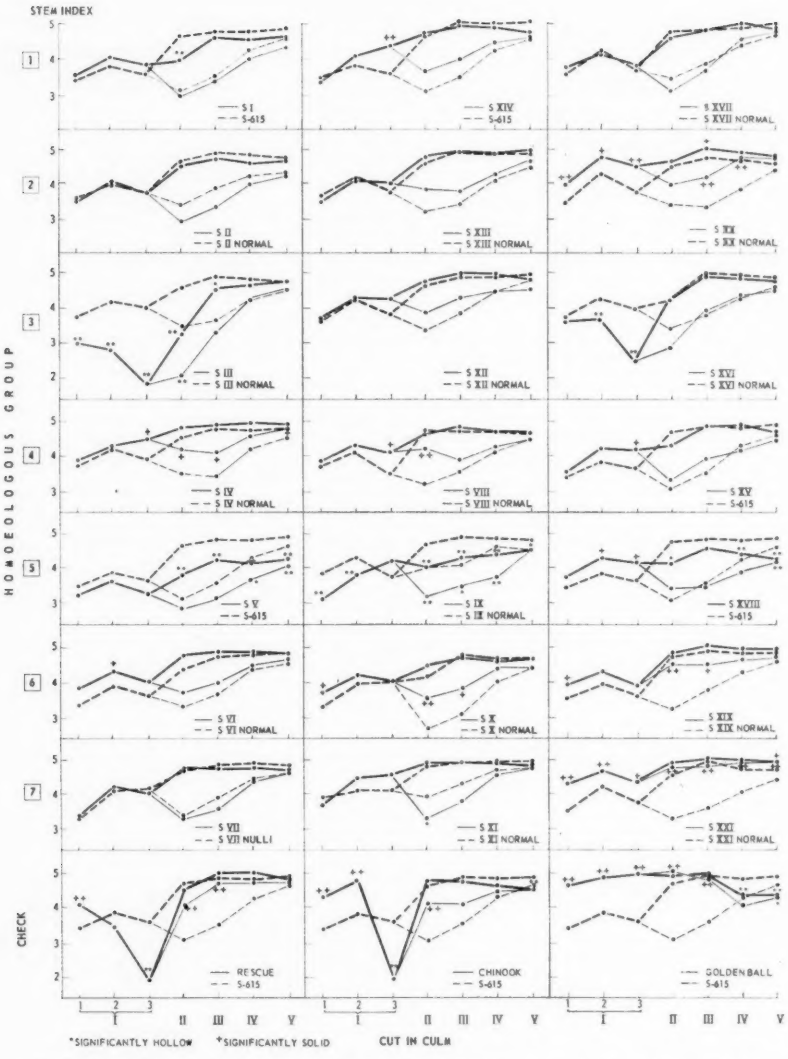

FIG. 1. Solid stem gradients in monosomics of S-615 and in check varieties. Numbers at the left of each row of graphs indicate the homoeologous group to which the missing chromosomes of the monosomics in the three graphs of the row belong.

I, both differences significant at the 1% level. Because no derived normal was available as a check it is impossible to know whether these deviations from S-615 were due to loss of the specific chromosome or whether they were characteristic of the particular backcross lines.

In homoeologous group 2 only SXX was more solid than normal. The effect was general over the whole culm. This indicates that chromosome XX of S-615 tends to suppress solid stem but II and XIII do not. Alternatively, II and XIII might carry dominant pith inhibitors and the loss of one of them could not be detected. However, the latter interpretation is incompatible with the F_2 results (4).

In homoeologous group 3, SIII and SXVI were much less solid in the top internode than normal. Their solid stem gradients were similar, but SIII was more extreme with hollowness extending down to internodes II and III. Thus, chromosomes III and XVI of S-615 promoted pith development in the upper part of the culm, and their genes are not fully effective in single dose. The solid stem gradient of SXII was different from those of other members of the group. Although it resembled those of group 4, none of its cuts was significantly different from normal.

All members of homoeologous group 4 were more solid than normal in the third cut of internode I. SIV and SVIII were more solid than normal in some of the special cuts of the lower internodes, too, but SXV was not. Chromosomes of this group tend to inhibit pith development in the lower part of the top internode, and SIV and SVIII tend to suppress it in the upper part of the lower internodes. However, the effect is weak.

Chromosomes of homoeologous group 5 are needed to produce solidness in the lower internodes, as SV, SIX, and SXVIII were less solid than normal at most of these points. Concerning the top internode, SV was normal, SIX was hollow, and SXVIII was solid. The latter fact probably accounts for SXVIII having a normal total solid stem index, the excess solidness of the top internode compensating for the greater hollowness of the bottom. This agrees with results of the F_2 test of chromosome XVIII (4).

All S-615 monosomics of homoeologous group 6 were slightly more solid than normal in the top internode. Both SX and SXIX were much more solid than normal in the special cut of internode II and somewhat more solid in the special cut of internode III. The same trend was evident at other special cuts of these monosomics and at all special cuts of SVI, but the data are not statistically significant. However, total solid stem index of standard cuts of SVI was higher than normal, significant at the 5% level, and total solid stem indices of special cuts of SX and SXIX were higher than normal, the former at the 5% level and the latter at the 1% level (Table I). It may be concluded that the chromosomes of homoeologous group 6 in S-615 tend to suppress pith production throughout the plant, and that XIX, the D-genome chromosome, is most effective.

The A- and B-genome chromosomes of homoeologous group 7 in S-615 probably have little effect on the pith content of the culm. The special cut in internode II of SXI was less solid than the corresponding location of SXI

normal (Table IV). Both SXI and SXI normal were more solid than S-615. The raw data showed that this was due to one of the lines from which both monosomic and normal selections were made. No explanation can be made on the basis of present evidence. The check for SVII was mainly nullisomic and it differed little from SVII; the nullisomic was more solid than S-615 in the third cut of internode I (Table II). SXXI was the most solid of all lines and varieties grown, its total solid stem index exceeding even that of the solid-stemmed durum, Golden Ball (Table I). The special cuts (Fig. 1 and Table IV) were almost completely solid, indicating that the lower internodes were filled with pith near the top in most culms examined. Chromosome XXI is probably the most important pith inhibitor in S-615.

Comparison of solid stem gradients of Rescue and Chinook (Fig. 1) with those of SIII and SXVI suggests that these varieties received genes from either chromosome III or XVI of their hollow-stemmed parents, Apex and Thatcher respectively. However, Rescue and Chinook are generally somewhat more solid than SIII and SXVI by standard cut and much more so by special cut (Table I). Therefore, it is probable that they also received genes from their hollow-stemmed parents that had less pith inhibition than their alleles in S-615. Golden Ball, as is usual when it is grown on irrigated land, tended to be hollow in the bottom two internodes, although very solid at other points. This suggests that either chromosome V or IX of Golden Ball is less effective in producing pith in the lower internodes than its homologue in S-615. The absence of the D genome in Golden Ball results in a lower proportion of pith inhibitors, and consequently in a very solid culm.

Discussion

The culm phenotype of the common wheat variety S-615 in a given environment is apparently the result of interaction of genes promoting pith development and genes suppressing it. Of genes for solid stem those on chromosomes of homoeologous group 3 produce pith in the top internode and to a lesser extent in the second, whereas those on chromosomes of homoeologous group 5 produce pith in the lower internodes. On the other hand, genes for hollow stem act throughout the culm. Chromosomes of homoeologous groups 4 and 6 inhibit pith development to some extent, and pith distribution is similar in monosomics within each group. Chromosomes XX and XXI, D-genome chromosomes of homoeologous groups 2 and 7 respectively, inhibit pith development although their group mates in the A and B genomes, with the possible exception of XI, have little effect.

Results of analysis of monosomic lines of S-615 are compatible with most of the observations on the F_2 hybrids of Chinese Spring monosomic lines by S-615 (4) and they answer some of the questions raised by that investigation. According to the F_2 test, chromosomes of homoeologous group 2 in Chinese Spring inhibit pith development, II weakly and XIII and XX strongly. The inhibition by XIII is due to a single gene for hollow stem in Chinese Spring that S-615 does not possess. The F_2 test did not give conclusive

evidence about chromosomes II and XX in S-615 but suggested that XX at least tends to produce hollowness; the monosomic test showed that chromosome XX of S-615 inhibits pith but II and XIII do not. Monosomic XIX and XXI F_2 lines produced such anomalous ratios that the most reasonable interpretation was that these chromosomes in S-615 tend to produce hollow culms; the monosomic lines of S-615 confirmed this interpretation. Chromosome VIII in Chinese Spring but not in S-615 was shown to produce a pithy culm; the monosomic test showed that S-615 chromosome VIII does not produce pith. The F_2 test failed to identify chromosomes in S-615 carrying genes for solid stem; the monosomic test showed them to be III and XVI for the top internode and V, IX, and XVIII for the bottom internodes. This supports the conclusion (9) that genes for solid stem are recessive.

This study and the monosomic F_2 analysis of solid stem in S-615 (4) suggest a slight revision of certain details of Yamashita's (13) theory of inheritance of solid and hollow stem in wheat, which could reconcile published researches that previously appeared to be contradictory. Yamashita proposed a comprehensive explanation of the genetics of solid stem in the genus *Triticum*. Somewhat simplified, the theory states that the diploid wheats have a gene for hollow stem, m_A, for which no alternative allele is known in the A genome of tetraploid and hexaploid wheats. The B genome has an allelic series of genes at locus M_B; genes for the most solid condition are dominant to alleles for lesser amounts of pith and epistatic to m_A. The D genome has a gene O_D, for hollow stem on chromosome *e* according to Kihara's designation (6, 13). This chromosome is the same as Sears' XX (7). It is epistatic to genes for solid stem at M_B. In addition, the A genome has a complementary gene, *C*, for pith in the lower internodes. It may be present in hollow-stemmed wheats, but is expressed only in the presence of genes for solid stem at the M_B locus.

The monosomic analysis confirms O_D on chromosome XX and the F_2 test (4) strongly suggests that m_A is on its homoeologue II. The third member of homoeologous group 2, XIII, has a gene for hollow stem in Chinese Spring but a null in S-615. If this chromosome bears the locus M_B, as seems probable, the gene for hollow stem is the active one and the gene for solid stem is a null which permits the effects of genes for solid stem at other loci to appear. In a tetraploid wheat, which lacks the pith inhibition of the D genome, the gene for hollow stem could be almost ineffectual in single dose, resulting in apparent dominance of solidness.

The complementary gene *C* for pith in the bottom internodes could be on either chromosome V or IX according to the monosomic test. As Okamoto (8)[3] has shown that IX is in the A genome, it probably carries gene *C*. Duplicates of *C* appear on V and XVIII, the other two members of homoeologous group 5. Complementary genes for pith in the top internode were demonstrated on chromosomes III and XVI of homoeologous group 3; XII does not have such a gene in S-615, but there may be varieties of wheat, especially tetraploids, in which it does.

[3]Cited with permission of the author.

The D genomes of Chinese Spring and S-615 have genes for hollow stem on chromosomes XIX and XXI in addition to XX already established. Not all hollow-stemmed hexaploid wheats have more than one, as may be calculated from data reported by Yamashita (13) and Matsumura (5). In their experiments pentaploid F_1 hybrids of solid-stemmed tetraploids and hollow-stemmed hexaploids were backcrossed to the respective tetraploid parent. The resulting offspring had 14 pairs of chromosomes of the A and B genomes and 0 to 7 univalents from the D genome. All 28-chromosome plants had solid culms. If only one of the D-genome chromosomes had an epistatic gene for hollow stem, all 35-chromosome plants would be hollow; if two of them produced such an effect, all 34- and 35-chromosome plants would be hollow. They found that all 35-chromosome plants were hollow, but in their combined data only 82% of the 34-chromosome plants were, a reasonable approximation to the 85.5% hollow expected on the basis of one such chromosome. The D genome tends to make hexaploid wheats hollow, but more than one chromosome may be involved.

If these changes in Yamashita's theory are valid, they would resolve a conflict regarding the relative importance of the A and B genomes in pith production in the wheat culm. Yamashita (13) stated that it is the B genome that produces pith. Yet Thompson (12) demonstrated that the difference between hollow-stemmed *T. monococcum* and solid-stemmed tetraploid wheats is due to genes in the A genome and not to the presence of the B genome. The triploid F_1 had solid stems. In the F_2, most of the plants of which had 28 chromosomes and none of which had fewer, approximately one plant in 15 had a hollow stem. This would be comprehensible on the basis that the tetraploids used had solid stems, and so lacked the active gene for hollow stem at locus M_B on chromosome XIII of the B genome; it would also have genes for solid stem on chromosomes of groups 3 and 5. In the absence of a D genome they would probably be dominant. If two of these genes are in the A genome and *T. monococcum* has the corresponding null alleles or weak ones, a dihybrid ratio of 1 hollow to 15 solid would be expected when segregation of genes in the A genome is normal. That is approximately what Thompson found. However, in crosses between varieties and species that have the same genes for solid stem on chromosomes of the A genome, presence or absence of the gene for hollow stem on chromosome XIII would be critical.

Other conflicting results may be explained on a similar basis. Putnam (10) found solid stem to be dominant and monohybrid in tetraploid crosses whereas Goytia y Angulo (3) found it to be recessive and dihybrid. Probably Putnam's varieties differed mainly in the hollow-stemmed varieties having the gene for hollow stem on chromosome XIII; Goytia y Angulo's hollow-stemmed variety either lacked two of the genes for solid stem such as those on chromosomes of homoeologous groups 3 and 5 or lacked one of them and had a gene for hollow stem on XIII.

The transgressive segregation that appeared in a few experiments might be explained on the basis that the amount and distribution of pith in the

culm is the result of genes tending to produce pith and those tending to make the culm hollow. Since many loci are available for gene differences, two varieties with similar amounts of pith may have quite different gene complements. In the F_2 generation a reassortment of genes could produce hybrids different from either parent. Thus, in the hybrid of Rivet (14″, upper internode filled at the top, intermediate at the base) and Chinese White (21″, upper internode thick-walled, hollow at the top, thin-walled, hollow at the base), Engledow and Hutchinson (2) found 16 plants out of 152 with top internodes completely filled with pith. The same authors (2) reported a tetraploid hybrid of *T. turgidum* × *T. polonicum* in which both had pith in the top internode but the distribution was different. Out of 522 F_2 plants, 129 were nearly as hollow as hollow-stemmed varieties of *T. aestivum*. They explained these discrepancies on the basis of differences in culm diameter affecting the relative pith content, although Biffen (1) found that culm diameter and pith were inherited independently. It is equally plausible that transgressive segregation observed in their experiment was due to reassortment of genes directly affecting pith development.

As Sears (11) has observed, the concept of dominance or recessivity as applied to diploid organisms is frequently not adequate to explain inheritance in polyploid wheats; for example, a deficiency may appear as dominant. The reason for this is exemplified in the monosomic studies of solid stem in S-615. Where two or three chromosome pairs are critical for either solid or hollow stem, dosage effects are shown in a reduction from four to three and from six to five respectively. In a given environment the amount and distribution of pith in the stem is determined by the balance of genes tending to make the stem solid and those tending to make it hollow. The loss of any one of them tends to produce the opposite phenotype.

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CYTOGENETICS OF SOLID STEM IN COMMON WHEAT

III. CULM MEASUREMENTS AND THEIR RELATION TO STEM SOLIDNESS IN MONOSOMIC LINES OF THE VARIETY S-615[1]

RUBY I. LARSON AND M. D. MACDONALD[2]

Abstract

Most monosomics of the solid-stemmed variety of common wheat, S-615, were shorter than normal. Many had thinner culms, but monosomics XIII and XVI had thicker culms. Although the effect of loss of homoeologous chromosomes on culm dimensions was generally similar in S-615 monosomics and Chinese Spring nullisomics, the most extreme member of each group was not always the same in the two varieties. The differences between solid stem in monosomics and normal lines of S-615 were due to loss of the chromosome affecting pith production and not to secondary effects caused by changes in culm dimensions. Nevertheless, within lines of genetically similar plants there was a small but consistent association between short culm and solidness, between thin culm at the tops of internodes I, III, and IV and hollowness, and between thin culm in the center and lower parts of internode I and solidness. This has a bearing on interpretation of data in monosomic analysis of solid stem.

Introduction

Monosomic analyses of solid stem in S-615, a variety of common wheat, *Triticum aestivum* L. *emend* Thell., showed that loss of certain chromosomes alters the amount and distribution of pith in the culm (4, 5). Loss of chromosomes in the variety Chinese Spring affects culm dimensions, most monosomics and nullisomics being shorter and thinner than normal, but at least one being taller and two coarser (7). The question arises whether the change in relative pith content is a result of altered growth rates in either length or diameter of the culm, or whether it is a direct specific effect of loss of genes determining pith development.

Conflicting opinions on the relation of solid stem to culm dimensions have been expressed. Engledow (2) observed segregates in the F_2 of the hybrid *T. polonicum* × *T. durum* var. Kubanka with pith patterns different from those of the parents. He thought that characteristics of the culm other than pith content influenced classification. Engledow and Hutchinson (3) observed transgressive segregation for solid stem in the hybrids of *T. turgidum* var. Rivet × *T. aestivum* var. Chinese White. In the F_2 generation some plants were more solid than Rivet, the solid-stemmed parent, and some had thinner culm walls than Chinese White, which they described as "essentially hollow". They suggested that the result might have been due to the effect of other straw characters on solidness. Biffen (1), contrariwise, on the basis of inheritance studies in a hybrid of *T. turgidum* × *T. aestivum* concluded that culm diameter, thickness of sclerenchyma wall, ridging of culm, and the arrangement of epidermal hairs segregated independently of pith content of the culm.

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Growth of the wheat culm proceeds by elongation from an intercalary meristem at the base of each internode. The order and degree of elongation and maturation of each internode is related to its rank in relation to the inflorescence, no two internodes being alike in this respect (6). Therefore, an investigation into the relationship between solid stem index, as a measure of pith content of the culm, and diameter and elongation of the culm would have to be specific as to location. This investigation was undertaken to determine to what extent, if any, solidness is related to culm dimensions.

Materials and Methods

Culm measurements were made on the same plants of lines of S-615 monosomic for each of the 21 chromosomes of common wheat that were grown and examined for solid stem at Lethbridge in 1954 (5). The test included as checks S-615 from regular stock and normal S-615 lines derived from lines monosomic for all chromosomes but I, V, XIV, XV, and XVIII. Monosomic lines are designated by the letter S followed by the number of the missing chromosome, e.g. SII, SIII, with the corresponding derived normals called SII normal and SIII normal. The check for SVII was mainly nullisomic, i.e. SNVII. Two solid-stemmed commercial varieties of common wheat derived from S-615, Rescue and Chinook, as well as the variety of *T. durum* Desf., Golden Ball, were included. Ten culms of each plot in each of four replicates were examined, except for one missing plot in each of SIII, SIV normal, and SX. Lengths of culms and culm internodes were measured with a rule graduated to .1 cm. Measurements of internodes were made between the centers of the nodes.

Diameters were measured at all points of cut, i.e. in the centers of all internodes and in the top internode (I) at 5 cm from each end, in internodes II and III at 2 cm from the top, and in internodes IV and V at 1 cm from the top. Because the culms are compressible and not perfectly circular, individual measurements were made to 0.1 mm only in spite of the fact that the micrometer calipers used were graduated to .01 mm.

Data on total culm length were analyzed by analysis of variance, and correlation coefficients between culm lengths and total solid stem indices for both standard and special cuts were calculated. As the loss of certain chromosomes affected the solid stem indices of the top and the lower internodes independently the percentage of the culm length represented by the top internode was analyzed by analysis of variance to test the possibility that differences were due to differential elongation. Plants with four internodes were analyzed separately from five-internode plants. SX had too few five-internode plants and Golden Ball too few four-internode plants for analysis of these respective categories.

The data on culm diameter at each point of cut except those of internode V were analyzed by analysis of variance. Correlation coefficients between diameter and solid stem index were calculated for each point of cut in internode I and for special cuts in internodes II, III, and IV within each line.

As the center cuts of internodes II, III, and IV and both cuts of internode V were usually solid, correlation coefficients between solid stem index and diameter were not calculated for these points. In Golden Ball only cut 1 in internode I and the special cut in internode IV were variable enough to justify calculations of correlation coefficients.

Results

Culm Length

Table I shows that no derived normal was significantly different from S-615 in culm length; no monosomic had greater culm length than S-615 but SI and SIX were just as long. These results may be compared with Sears' observations on nullisomics and monosomics of Chinese Spring (7). Sears observed total plant height, not just culm length.

None of the monosomic lines of S-615 in homoeologous group I was significantly different from S-615 or the derived normal. Sears found that the nullisomics of this group in Chinese Spring were reduced in height.

TABLE I

Culm length in cm of S-615 monosomics, of their derived normals, and of check varieties. Lethbridge, 1954

Line	Homoeologous group	Monosomic	Derived normal
SI	1	100.4	—
SXIV		95.3	—
SXVII		94.7	97.3
SII	2	93.6	97.2
SXIII		96.2	100.1
SXX		84.8**	100.1
SIII	3	91.8*	99.8
SXII		94.2	98.6
SXVI		88.8**	98.4
SIV	4	87.9**	98.4
SVIII		93.6	98.9
SXV		96.6	—
SV	5	92.1*	—
SIX		100.6	100.5
SXVIII		93.8	—
SVI	6	87.9*	95.6
SX		89.6**	101.6
SXIX		92.6	98.4
SVII	7	92.6*	—
SNVII		89.4**	—
SXI		92.6	96.5
SXXI		89.4**	98.8
S-615		—	99.9
Rescue		—	93.3*
Chinook		—	92.4*
Golden Ball		—	109.6††
L.S.D. .05 = 6.4			
L.S.D. .01 = 8.5			

NOTE: The monosomics are compared with the corresponding normals when these are present and with S-615 when they are not; normals are compared with S-615.
*Shorter than check.
†Longer than check.

In homoeologous group 2 SXX was shortest, SII and SXIII being only a few centimeters shorter than their derived normals. In Chinese Spring nullisomics nulli-XX was taller than the other two.

As in Chinese Spring, the loss of chromosome XVI of S-615 shortened the plant more than the loss of either of the other members of homoeologous group 3. Loss of XII shortened the culm least, whereas loss of III resulted in a plant significantly shorter than normal at the 5% level.

In homoeologous group 4 SIV was the only line significantly shorter than normal. Sears found nulli-VIII in Chinese Spring to be shortest.

In group 5 only SV was significantly shorter than S-615, but SXVIII was only 2 cm longer than SV, so they were more alike than either was like SIX. SIX was of normal length, although both the nullisomic and monosomic of chromosome IX of Chinese Spring were taller than normal.

As in Chinese Spring, loss of chromosomes of homoeologous group 6 in S-615 resulted in shorter-than-normal plants. Both SVI and SX were very short but SXIX was not significantly so. In Chinese Spring nulli-XIX was tallest of the nullisomics.

All monosomics of homoeologous group 7 in S-615 were shorter than the parent variety, although SXI was not significantly different from SXI normal. SXXI was shortest, equal to SNVII. This agrees with Sears' observations that in Chinese Spring nulli-XXI was shorter than the other nullisomics of group 7.

Relation of Total Solid Stem Index and Culm Length

Coefficients of correlation between culm length (Table I) and total solid stem index for both standard and special cuts (5) are given in Table II. Among the 36 values of r for derived normals and normal checks 9 were positive and 27 negative, showing a general trend in the negative direction. This means that within normal lines the taller plants tended to be less solid than the shorter ones. Although two values of r significant at the 5% level can be expected out of this number on the basis of chance, the results of SIII normal require interpretation. Replicate 4 of that line was from a different seed source than the other three replicates. It had a mean culm length of 106.8 cm compared with 97.5 for the other replicates and 99.9 for S-615 and had a mean total solid stem index for standard cuts of 28.6 compared with 31.7 for the other three replicates and 30.2 for S-615; its index for special cuts was 24.1 compared with 29.1 for the other replicates and 26.5 for S-615. The low solid stem index was not due to the loss of a portion of chromosome III causing it to resemble its SIII parent, as its pith gradient was similar to S-615 but was less solid. Neither was it likely due to the replicate, as replicate 4 was generally a little more solid than replicates 1 and 3. Omitting replicate 4, the value of 4 was $-.264$ for standard cuts and $-.190$ for special cuts, neither significant for 28 degrees of freedom. Therefore, the most likely explanation of the high correlation coefficient is that the SIII normal plants grown in replicate 4 were genetically different from those of other replicates for long culm and hollowness.

TABLE II

Correlation coefficients of culm length and total solid stem index within monosomic lines of S-615, their derived normals, and check varieties. Lethbridge, 1954

Line	Monosomic		Derived normal	
	Standard cut	Special cut	Standard cut	Special cut
SI	−.329*	−.055	—	—
SXIV	−.002	−.179	—	—
SXVII	−.026	−.031	−.031	−.038
SII	−.027	−.028	.037	.085
SXIII	−.181	−.120	−.285	−.104
SXX	−.076	.137	−.102	−.120
SIII	−.040	−.013	−.417**	−.422**
SXII	−.363*	−.418**	.250	.043
SXVI	.131	.117	−.312*	−.217
SIV	−.103	−.093	−.329	−.260
SVIII	−.305	−.362*	.030	−.084
SXV	.095	.083	—	—
SV	.229	.254	—	—
SIX	.181	.213	−.110	−.076
SXVIII	.208	.432**	—	—
SVI	−.425**	−.472**	.247	.082
SX	.085	.117	−.233	−.279
SXIX	.210	.329*	−.070	.027
SVII	.324*	.239	—	—
SNVII	.025	−.231	—	—
SXI	−.210	−.053	−.009	−.135
SXXI	−.202	−.208	−.014	.029
S-615	—	—	−.143	−.011
Rescue	—	—	−.271	−.103
Chinook	—	—	−.193	−.386*
Golden Ball	—	—	−.089	−.149

NOTE: SIII, SIV normal, and SX have 28 degrees of freedom, all others 38.

D.f. 38	D.f. 28
r .05 = .312	r .05 = .361
r .01 = .403	r .01 = .463

In the monosomic lines the strong negative correlations between solid stem index and culm length in SVI and SXII could be due to their monosomics being difficult to distinguish from normals by inspection. The monosomics have a tendency to be both short and slightly more solid than normal, so in a population made up of both monosomics and normals the correlation is as expected. The strong positive correlation in SXVIII between solid stem in special cuts and culm length cannot be explained on a similar basis, as monosomics of this line were selected morphologically. All members of homoeologous group 5 showed a positive correlation between solid stem and culm length, contrary to the trend for negative correlations in derived normals and exclusively negative correlations in the varietal checks. Yet it could not be due to mixtures of normals in the monosomic population as these monosomics are morphologically distinguishable. Further, although SV and SXVIII tend to be short, SIX is fully as long as S-615 or its derived normal. As this trend in correlation runs counter to the negative trend in the cytogenetically normal lines, it is physiologically and genetically important.

Comparisons between monosomic lines for solid stem (5) and culm length (Table I) show that losses of chromosomes that affect culm length in the same way are not consistent in their effect on solid stem. SIII, SIV, SV, SVI, SVII, SNVII, SX, SXVI, SXX, and SXXI were all shorter than normal. Of these SIV, SVI, SX, SXX, and SXXI were more solid than normal according to total solid stem index for either standard or special cuts or both; SIII, SV, and SXVI were more hollow; SVII and SNVII had normal solid stem indices. Further, SIX was hollow and SXIX solid but neither was significantly shorter than its derived normal.

Proportionate Lengths of Top Internode

The top internode of the wheat culm is approximately half the total culm length, and each succeeding lower internode is about half the length of the one above. Monosomics III and XVI in S-615 were found to be less solid in the top part of the culm and V, IX, and XVIII less solid in the lower internodes than normal (5). Conceivably this could be due to differences in proportionate rates of elongation of the top internode and the others.

TABLE III

Percentage of culm length represented by the top internode in monosomic lines of S-615, their derived normals, and check varieties. Lethbridge, 1954

	5-internode plants		4-internode plants	
Line	Monosomic	Derived normal	Monosomic	Derived normal
SI	45.8	—	50.4*	—
SXIV	49.4	—	53.8	—
SXVII	48.8	48.3	53.0	55.5
SII	46.8	46.9	52.4	52.6
SXIII	48.3	48.0	51.9	51.3
SXX	49.0	47.6	53.6	55.4
SIII	48.4	48.0	55.5	53.3
SXII	50.4	47.5	54.7	52.2
SXVI	46.6	46.5	53.8	52.4
SIV	44.1**	48.4	50.0	49.6*
SVIII	47.2	48.1	52.3	54.0
SXV	45.9	—	52.7	—
SV	46.2	—	52.6	—
SIX	46.9	48.0	52.4	52.1
SXVIII	48.5	—	51.8	—
SVI	52.2	50.9	54.0	55.0
SX	—	46.6	53.6	52.1
SXIX	47.8	47.5	52.8	53.3
SVII	49.8	—	54.4	—
SNVII	53.2††	—	56.4	—
SXI	48.3	50.5	53.1	52.7
SXXI	45.8	46.8	51.6	53.6
S-615	—	48.3	—	53.6
Rescue	—	51.2	—	54.5
Chinook	—	54.2††	—	58.2††
Golden Ball	—	48.8	—	—
L.S.D. .05	3.3		3.2	
L.S.D. .01	4.3		4.3	

*Less than check.
†More than check.

The percentage of culm length represented by the top internode is summarized in Table III. Among five-internode plants no derived normal differed significantly from S-615. The top internode of SIV was proportionately shorter than either its derived normal or S-615. In SVI it was longer than S-615 but not longer than its derived normal. SNVII had a top internode proportionately longer than that of either SVII or S-615. In the four-internode category, both SIV and SIV normal had a relatively shorter top internode than S-615. The top internode of Chinook was proportionately the longest in both five-internode and four-internode groups.

The percentage of culm represented by the top internode was remarkably constant within the four- and within five-internode classes of plants in the variety S-615. Where differences occurred they seemed to be unassociated with a differential effect of loss of a critical chromosome on solid stem index of the whole culm or part of it. The monosomics of homoeologous groups 3 and 5, which were less solid than normal in top and bottom parts of the culm respectively, showed no deviation from S-615 in proportionate lengths of the top internode. The long top internode of Chinook suggests that proportionate length of top internodes is a varietal character.

Culm Diameters

The data on culm diameters at all points of cut except those of internode V, which was not present in all plants, are given in Table IV for the top internode and in Table V for the lower internodes. Most derived normals were not significantly different from S-615 at many points. SX normal was thicker throughout and SXVI normal was thicker in the bottom internodes than S-615.

Monosomics of S-615 may be compared with nullisomics of Chinese Spring (7) within homoeologous groups.

The S-615 monosomic lines of group 1 had culms of essentially the same diameter as those of S-615. The Chinese Spring nullisomics of this group had culms thinner than normal. However, as nullisomics are usually much more extreme in any character than the corresponding monosomics it is possible that the nullisomics of S-615 may be similar to those of Chinese Spring. There was no evidence of reduced diameter in the monosomic.

In group 2 of S-615 only SXIII had a thicker culm than normal. This contrasts with Chinese Spring in which the culm of nulli-II was thickest, nulli-XX was less so, and nulli-XIII was essentially normal.

In group 3 only SXVI was different from normal. It was thicker than S-615 at cuts 1 and 2 of internode I and at both cuts in internode II. As SXVI normal had a greater diameter than S-615, SXVI was thicker than its derived normal at only cut 1, internode I. In Chinese Spring nulli-III was thinner than normal but nulli-XII and nulli-XVI were essentially normal in diameter.

In group 4 only SVIII was thinner throughout than either S-615 or its derived normal. In Chinese Spring both nulli-IV and nulli-VIII had culms much thinner than normal Chinese Spring.

All S-615 monosomics of group 5 had thinner culms than S-615. SIX contrasted strongly with its derived normal, the culms of which were thicker than those of S-615. These results agree with those of Chinese Spring in which all group 5 nullisomics had thin culms.

Monosomics of group 6 had generally thinner culms than normal. Diameters of SVI culms were consistently less than those of S-615 and its own derived normal except at cut 3, internode I. However, only cuts in the lower internodes were individually significant, and then only when compared with S-615. SX was thinner than its derived normal, which was significantly thicker than S-615. SXIX was significantly thinner in the lower internodes than either SXIX normal or S-615. Sears found all nullisomics of Chinese Spring in this group had slender culms.

In group 7 only the nullisomic, SNVII, differed significantly from normal at many points. Its culms were thinner than those of S-615 and SVII in the bottom internodes. SXI was thinner than either S-615 or SXI normal at cut 1, internode I. As Sears did not mention culm diameter in this group it is assumed that the diameters of the culms of Chinese Spring nullisomics VII, XI, and XXI were normal.

TABLE IV

Culm diameters (mm) at points of cut in the top internode of S-615 monosomics, their derived normals, and check varieties. Lethbridge, 1954

Line	Cut 1		Cut 2		Cut 3	
	Mono	Normal	Mono	Normal	Mono	Normal
SI	1.90	—	2.33	—	2.34	—
SXIV	1.81	—	2.25	—	2.26	—
SXVII	1.84	1.89	2.30	2.31	2.29	2.26
SII	1.82*	1.90	2.28	2.34	2.26	2.24
SXIII	1.94	1.87	2.38	2.32	2.33	2.28
SXX	1.90	1.86	2.32	2.30	2.31	2.26
SIII	1.90	1.84	2.32	2.27	2.33	2.25
SXII	1.81	1.84	2.25	2.31	2.26	2.24
SXVI	2.01††	1.89	2.39	2.35	2.34	2.28
SIV	1.83	1.82	2.23	2.21	2.24	2.20
SVIII	1.71**	1.87	2.13**	2.32	2.12**	2.28
SXV	1.79	—	2.24	—	2.29	—
SV	1.70**	—	2.10**	—	2.14*	—
SIX	1.74**	1.86	2.20*	2.30	2.16	2.23
SXVIII	1.69**	—	2.12**	—	2.06**	—
SVI	1.82	1.85	2.27	2.31	2.28	2.25
SX	1.87	1.94†	2.26**	2.42††	2.18**	2.33
SXIX	1.80	1.84	2.23	2.32	2.19	2.26
SVII	1.86	—	2.32	—	2.26	—
SNVII	1.82	—	2.23	—	2.25	—
SXI	1.75*	1.85	2.23	2.31	2.21	2.25
SXXI	1.83	1.79	2.24	2.21	2.26	2.24
S-615	—	1.84	—	2.29	—	2.26
Rescue	—	1.78	—	2.24	—	2.35
Chinook	—	1.79	—	2.12**	—	2.20
Golden Ball	—	2.14††	—	2.95††	—	2.76††
L.S.D. .05	.08		.10		.10	
L.S.D. .01	.11		.13		.14	

*Smaller diameter than check.
†Larger diameter than check.

TABLE V

Culm diameters (mm) at points of cut in the lower internodes of S-615 monosomics, their derived normals, and check varieties. Lethbridge, 1954

	Standard cut						Special cut					
	II		III		IV		II		III		IV	
Line	Mono	Normal	Mono	Normal	Mono	Normal	Mono	Normal	Mono	Normal	Mono	Normal
SI	2.95	—	2.83	—	2.75	—	2.88	—	2.79	—	2.67	—
SXIV	2.90	—	2.76	—	2.67	—	2.74	—	2.73	—	2.69	—
SXVII	2.90	2.87	2.78	2.77	2.68	2.69	2.84	2.87	2.80	2.76	2.64	2.64
SII	2.84	2.92	2.78	2.76	2.62	2.66	2.85	2.88	2.80	2.78	2.62	2.62
SXIII	3.02†	2.87	2.88	2.82	2.87	2.74	2.95†	2.81	2.90	2.81	2.84	2.70
SXX	2.94	2.90	2.77	2.85	2.69	2.76	2.80	2.79	2.79	2.83	2.67	2.73
SIII	2.97	2.85	2.80	2.78	2.68	2.76	2.96	2.84	2.80	2.76	2.63	2.74
SXII	2.84	2.89	2.74*	2.86†	2.65	2.76	2.80	2.88	2.71*	2.83	2.62	2.75†
SXVI	2.98	2.99†	2.81*	2.92††	2.74	2.78†	2.97	2.96	2.82	2.88††	2.70	2.74
SIV	2.97†	2.82	2.76	2.70	2.62	2.65	2.80	2.69*	2.70	2.69	2.63	2.66
SVIII	2.71*	2.88	2.55**	2.77	2.48**	2.69	2.67**	2.86	2.58**	2.80	2.43**	2.64
SXV	2.80	—	2.74	—	2.69	—	2.74	—	2.71	—	2.64	—
SV	2.69*	—	2.58**	—	2.45**	—	2.65**	—	2.52**	—	2.45*	—
SIX	2.74**	2.96†	2.69**	2.87†	2.58	2.76	2.68**	2.88	2.58**	2.82	2.54*	2.73
SXVIII	2.69*	—	2.62**	—	2.59	—	2.60**	—	2.58*	—	2.60	—
SVI	2.81	2.86	2.66	2.74	2.50	2.62	2.68	2.80	2.66	2.71	2.50	2.62
SX	2.84*	2.98†	2.64**	2.86†	2.57*	2.72	2.85	2.97†	2.72*	2.84†	2.52*	2.68
SXIX	2.76*	2.92	2.60**	2.80	2.50*	2.64	2.66**	2.84	2.59**	2.76	2.46	2.60
SVII	2.94	—	2.77	—	2.64	—	2.85	—	2.70	—	2.59	—
SNVII	2.75	—	2.57**	—	2.42**	—	2.70*	—	2.62	—	2.40**	—
SXI	2.85	2.80	2.69	2.76	2.61	2.70	2.82	2.75	2.69	2.76	2.58	2.68
SXXI	2.91	2.85	2.80	2.76	2.70	2.78†	2.74	2.74	2.75	2.76	2.68	2.74
S-615	—	2.83	—	2.76	—	2.64	—	2.84	—	2.72	—	2.60
Rescue	—	2.91	—	2.80	—	2.75	—	2.75	—	2.73	—	2.65
Chinook	—	2.79	—	2.70	—	2.66	—	2.70	—	2.72	—	2.60
Golden Ball	—	3.44††	—	3.48††	—	3.33††	—	3.26††	—	3.36††	—	3.36††
L.S.D. .05		.13		.10		.14		.13		.12		.15
L.S.D. .01		.18		.13		.19		.17		.16		.20

*Smaller diameter than check.
†Larger diameter than check.

TABLE VI

Correlation coefficients of solid stem index and culm diameter at designated points of cut on culms of S-615 monosomics, their derived normals, and check varieties. Lethbridge, 1954

	Top internode (I)						Lower internodes, special cut					
	Cut 1		Cut 2		Cut 3		II		III		IV	
Line	Mono	Normal	Mono	Normal	Mono	Normal	Mono	Normal	Mono	Normal	Mono	Normal
SI	−.012	—	.000	—	−.350*	—	.174	—	.294	—	.298	—
SXIV	−.160	—	.000	—	.042	—	−.233	—	.313*	—	.017	—
SXVII	−.204	.211	.050	−.241	.050	−.249	.209	.282	.394*	.342*	.248	.179
SII	−.051	−.074	−.305	−.202	−.230	.026	.000	−.273	.187	.069	.410**	.160
SXIII	.059	.211	−.026	−.206	−.093	−.057	.028	−.099	.173	.532**	.545**	.444**
SXX	.200	−.310	.143	−.406**	.189	−.281	.164	−.066	.287	−.173	.147	.312*
SIII	.054	.325*	−.309	.060	.032	.063	−.099	−.284	.220	.469**	.326	−.044
SXII	.054	.158	.043	−.230	.096	−.087	.189	.237	.057	−.102	.380*	.064
SXVI	.022	.174	−.401*	−.029	−.245	.091	−.287	−.139	.008	.184	.107	.464**
SIV	.105	.356	.241	.021	−.247	.265	.135	.184	.385*	.296	.162	.378*
SVIII	.246	−.079	−.396*	−.170	−.131	−.136	−.242	−.042	.013	−.041	.144	.118
SXV	.129	—	−.164	—	−.201	—	.322*	—	.332*	—	.196	—
SV	.038	—	−.270	—	−.202	—	.127	—	.116	—	.267	—
SIX	.322	.210	−.205	−.074	−.061	−.038	−.073	−.041	.278	.374*	.320*	.069
SXVIII	.141	—	−.349*	—	−.112	—	.240	—	.438**	—	.172	—
SVI	−.452**	−.239	−.576**	−.293	−.509**	−.216	−.161	−.018	−.187	.396*	−.267	.251
SX	.247	.120	−.216	−.124	.161	.154	−.039	.015	.174	.287	−.030	.135
SXIX	−.037	−.061	−.466**	−.154	−.190	−.127	.112	−.314	.401*	.405**	.258	.418**
SVII	.118	—	−.157	—	−.083	—	−.067	—	.389*	—	.199	—
SNVII	−.115	—	−.316*	—	−.034	—	−.030	—	.254	—	.068	—
SXI	.111	−.042	−.205	.110	.012	−.116	−.112	−.006	.270	.247	.129	.478**
SXXI	.358*	.192	.312*	−.341*	.231	−.101	.210	−.010	.374*	.175	.559**	.371*
S-615	—	−.030	—	−.148	—	−.126	—	−.236	—	.228	—	.186
Rescue	—	.266	—	−.474**	—	−.288	—	.131	—	.303	—	.185
Chinook	—	−.132	—	.237	—	.047	—	.046	—	.313*	—	−.136
Golden Ball	—	.142	—	—	—	—	—	—	—	—	—	−.285

NOTE: SIII, SIV normal, and SX have 28 degrees of freedom, all others 38.

D.f. 38	D.f. 28
r .05 = .312	r .05 = .361
r .01 = .403	r .01 = .463

The durum variety, Golden Ball, had much thicker culms than S-615, but Rescue and Chinook were generally similar to their parent, S-615.

Loss of chromosomes of most homoeologous groups in S-615 affected culm diameter in the same way as Sears found it in nullisomics of Chinese Spring, but relative effects among the deficiencies within a group were not always the same, notably in group 2. Further, certain nullisomics of Chinese Spring had thin culms but monosomics in S-615 had not, presumably because the loss of a pair of chromosomes is more drastic than the loss of one member of a pair.

Homoeologous group 3 had a different effect on culm diameter in S-615 from that in Chinese Spring (7). In Chinese Spring, nulli-III had thinner culms than normal. In S-615 the only monosomic affected was XVI and its culm was thicker than that of S-615. Only at cut 1 in internode I was it thicker than its derived normal, but the derived normal was thicker than S-615. It may be that this line has retained genes for thick culm from Chinese Spring from which it was derived by backcrossing, and which has thicker culms than S-615. On the other hand, the loss of chromosome XVI may cause a thick culm in this variety. The derived normal may have lost a portion of chromosome XVI too small to detect cytologically, the loss of which produced the deficiency-XVI effect on culm diameter. This question cannot be resolved on the basis of the present data.

Relation of Solid Stem Index and Culm Diameter at Each Point of Cut

Table VI gives the coefficients of correlation between solid stem index and culm diameter for each cut in the top internode and for special cuts in internodes II, III, and IV. There was no consistent difference between monosomic and normal lines in either direction or significance of correlations. There was a trend toward positive correlations (a few significant) at cuts 1, III (special), and IV (special), negative correlations at cut 2 and possibly 3, with no trend apparent at II (special).

The correlations significant at the 5% level are probably not very important except where they are consistent within a line, but no more than one or two at the 1% level could be expected on the basis of chance. SVI exhibited negative correlations throughout, although its derived normal follows the general trend except for cut 1. Table VI indicates that thin culms are most solid, thick ones least, and that the trend is strongest in the top internode. As SVI had thin culms (Tables IV and V) and tended to be solid (5), and as the population consisted of both normal and monosomic plants which cannot be distinguished from one another by inspection, the correlation is probably a reflection of these properties of the bimodal population. A similar situation was observed in the correlations of solid stem and culm length in this line.

The large number of positive correlations significant at the 1% level in the lower internodes agrees with the general trend for that portion of the culm. As there was no difference between monosomic and normal lines generally in this regard it can be postulated that the relation is inherent in the response

of the lower part of the culm to environment; those conditions that lead to vigorous growth in that region also cause pith to develop well.

The loss of various chromosomes affects both solid stem index (5) and culm diameter (Tables IV and V). Chromosomes of homoeologous group 1 appear not to influence either character greatly. In group 2 SXIII had a thick culm and SXX was solid. The only point at which the solid stem and large diameter were associated was at cut II (special) in SXIII. In homoeologous group 3, SIII and SXVI were hollow in the top and had thick culms, the two characters being frequently associated; SXII had a culm slightly more solid than that of S-615 and its culm tended to be slender at cut III (special). In group 4 monosomics IV and VIII had slender culms, which were associated with solid stem at several points in SVIII. All monosomics of group 5 had thin culms. This was associated with either a solid or a hollow top internode and with hollow lower internodes. Culms of monosomics of homoeologous group 6 had slender lower internodes. These were associated with solid, hollow, or normal culms. There was no consistent association among the characters of monosomics in group 7, although both solid stem index and culm diameter were affected.

Clearly, loss of a chromosome may influence both solid stem index and culm diameter, but there is no necessary association between the two; a thin culm may be solid, hollow, or like S-615. The same is true of thick culms and culms with diameters like those of S-615.

Discussion

Comparison of the effect of loss of a chromosome on solid stem index and on other culm measurements showed that the influence of critical chromosomes on solid stem is specific and not a secondary result of gene action that primarily governs culm growth. This was demonstrated by three types of observation. Culms of monosomics shorter than normal may be more or less solid than S-615 or may resemble it; loss of chromosomes of homoeologous groups 3 and 5, which influence the pith content of upper and lower parts of the culm differentially, did not alter the relative length of the top internode with respect to the rest of the culm; finally, there was no consistent association of diameter and solid stem index in comparisons between monosomic lines critical for solid stem.

However, within lines of genetically similar plants there were small but fairly consistent correlations between culm dimensions and solid stem. Short culms tended to be more solid than long ones; the top of internode I and the region just below the top node of each of internodes III and IV tended to be most solid when they were thickest but the center and lower parts of internode I were hollowest when they were thickest.

These observations have important implications in interpretation of solid stem data. Short monosomic populations that are just significantly more solid than normal may be merely reflecting the result of decreased elongation, but short monosomics that are just hollow at the point of significance have

gone counter to the prevailing influence of elongation and must be considered seriously. Correspondingly, lines with thin culms may be expected to be less solid in the tops of internodes I, III, and IV, and more solid in the lower parts of internode I. If they follow that trend to a slight degree, it may mean little, but if they reverse the trend to the same degree, the chromosome concerned must have a direct effect on pith production. Thick culms would show opposite trends.

The physiological significance of the relation of pith content to culm dimensions may be considered briefly. Skoog (8) has shown that low concentrations of indoleacetic acid favor cell division and high concentrations favor cell elongation in tobacco pith in vitro. In the wheat variety S-615, the top internode is the longest and the least solid of the internodes. As it is nearest the inflorescence it might be expected to receive the highest auxin concentrations from that source (6). The internodes become progressively shorter toward the root, and therefore probably have received smaller quantities of auxin. Within a population of genetically similar plants, those that elongate most, presumably under the influence of auxin, are least solid, probably because there are too few pith cells to fill the lengthened lumen. However, the upper parts of internodes I, III, and IV (V was too solid to test) were most solid in culms of the greatest diameter. The top parts of the internodes are laid down first by the intercalary meristem when the inflorescence is small and before the culm has begun to elongate greatly. This is the period of cell division, which is probably favored by the low concentration of auxin from the immature inflorescence. Rapid cell division would result in a thicker culm and an increased number of pith cells.

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DORMANCY STUDIES IN SEED OF AVENA FATUA

I. THE POSSIBLE ROLE OF GERMINATION INHIBITORS[1]

M. BLACK

Abstract

When aqueous extracts of hull and caryopsis of *Avena fatua* are chromatographed, two inhibitory regions can be detected. These two inhibitors appear to be present in the hull and caryopsis in approximately equal amounts per unit weight of tissue. Evidence has been brought forward to show that the inhibitory effect of the hull in intact dormant seeds cannot be ascribed solely to the inhibitors present in the hull, nor does interference with gaseous exchange by this tissue account for its effect. Experiments have shown that isolated caryopses from dormant seeds germinate only when in contact with liquid water. Evidence from several experiments indicates that the hull may prevent leaching of inhibitors from the caryopsis which then allows of germination. It has been shown that inhibitors very readily diffuse out of the caryopsis on moist filter paper and that this process is retarded by the hull. Treatment with high oxygen concentrations, which promotes germination of very dormant seeds, appears to result in a reduction of inhibitor level before visible germination. In the induction of secondary dormancy under anaerobic conditions there is a marked increase in the total inhibitor level of the caryopses.

Introduction

In common with seeds of many species, those of *Avena fatua* exhibit a period of dormancy during which no germination occurs even under apparently favorable conditions of water supply, temperature, etc. However, dormancy is slowly lost during "afterripening" and germination then takes place spontaneously under the forementioned conditions.

Although the physiological basis for the maintenance of seed dormancy is not fully understood, it is known that in many seeds the structures enclosing the embryo are of considerable importance. In some cases, the seed coat acts as a barrier to water uptake; in others, the enclosing structures are thought to impede oxygen entry. An effect of the enclosing structures can be seen in seeds of *Avena fatua* where caryopses removed from the hull germinate whereas intact non-afterripened seeds do not. Further, it is known that an incision in the true seed coat promotes germination of dormant seeds (1, 7). These effects have never been satisfactorily analyzed.

In recent years increasing attention has been paid to the part that naturally occurring inhibitors might play in dormancy phenomena, in both seeds and other organs. For seeds of a considerable number of species, the maintenance of dormancy has been ascribed to these inhibitory substances which have been found in the embryo and in other seminal tissues (e.g. 4). The possibility that naturally occurring inhibitors may be involved in the regulation of dormancy in *Avena fatua* has never been investigated.

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In this project, these aspects of dormancy in this seed have been examined. Particular attention has been paid to the detection of germination inhibitors and to the part they might play in dormancy.

In addition to its intrinsic biological interest, dormancy studies in this species are of considerable practical importance. It is partly due to this property of the seeds that the species is such a troublesome weed, especially in wheat-growing areas.

Materials and Methods

Seeds of *Avena fatua* were collected in a cultivated field near Saskatoon. Dormant seeds were obtained in subsequent years from this same collection which was grown under open field conditions. Seeds from this stock after-ripen during approximately two months; in the dormant condition, germination can be secured by removing the hull. A sample of highly dormant seed, in which hull removal is ineffective, was kindly supplied by D. H. Leighty of Montana State College. This strain, called 9A, breeds true phenotypically. We have obtained seeds from this original stock by cultivation in the greenhouse and in the field.

Germination Methods

Seeds were tested for germination in 9-cm petri dishes on two filter papers moistened with 3.5 ml of antibiotic solution. This solution, composed of 20 p.p.m. neomycin and 250 p.p.m. candidin, was effective in preventing bacterial and fungal growth. Germinations were carried out at 20° C in a dark cabinet maintained at 100% relative humidity.

Extraction and Chromatographic Techniques

Material was homogenized for approximately 30 minutes in distilled water. The resultant homogenate was stirred for 5 hours at approximately 5° C and then kept in a refrigerator for 16 hours. After filtration, the extract was concentrated under reduced pressure (<32° C) and during this operation the extract was centrifuged several times to remove starch and proteinaceous particles. When reduced to a small volume (0.5–1.0 ml) the extract was frozen for 16 hours and centrifuged once more, to obtain a clear, light-brown solution.

Extracts were "strip-loaded" onto Whatman No. 1 chromatographic paper. The descending method of chromatography was employed using a solvent composed of 99 parts 70% aqueous isopropanol:1 part ammonia (sp. gr. 0.9). After the solvent had reached a distance of approximately 30 cm from the base line, the paper was dried quickly in a cold air current.

Bio-assay Method

Assay for inhibitors was generally carried out using a non-light-requiring variety of lettuce seed (New York 515); the sensitivity of seeds of this species to inhibitors is well known. The chromatograms were cut horizontally into equal sections, usually 15 in number. Each strip was then placed in a small

petri dish and moistened with distilled water, the amount being dependent on the area of the strip. Approximately 60 lettuce seeds were placed on each strip and maintained in darkness at 20° C for 24 hours when the germination percentages were determined. A few bio-assays with wild oat caryopses were carried out using the same general method.

Experimental Results

It has been shown by earlier workers (1) that the germination of dormant seeds of *Avena fatua* can be brought about by removing the hull (lemma and palea) which is normally closely adherent to the caryopsis. We have confirmed this for certain "strains" of seed, others being so dormant that the treatment is without effect. Where removal of the hull is beneficial, the magnitude of the effect depends on the degree of afterripening.

There is presently no satisfactory explanation for the inhibitory effect of the hull. Two suggestions have been considered, viz. (*a*) that the hull impedes gaseous exchange, either oxygen entry or carbon dioxide escape, (*b*) that water uptake is retarded by the hull.

Studies by other workers (5) exclude the latter possibility. Furthermore, we have found that the uptake of water by the caryopsis is not limited by the presence of the hull.

TABLE I

The effect of various operations on the hull

Treatment	Percentage germination
Intact with hull	40
Hull loosened, half palea removed	30
Embryo exposed	50
Hull loose, seed coat cut near embryo	75
Isolated caryopses	83

The results given in Table I of an experiment designed to test critically the gaseous exchange hypothesis show that this, too, must be rejected. It has been found repeatedly that when the caryopsis is exposed to air by removing the palea, and the lemma loosened, there is no marked increase in germination of dormant seeds. This result receives corroboration from other experiments which are described below. The inhibitory effect of the hull cannot, therefore, be attributed to a prevention of gaseous exchange. However, the fact that an incision in the seed is markedly beneficial to germination, even in the presence of hull, may indicate that the true seed coat, when intact, impedes gas exchange. This will be considered in subsequent experiments.

A further possibility which might account for the inhibitory effect of the hull is that this tissue contains germination inhibitors. We have found that inhibitors are, indeed, present in the hull.

Figure 1 shows the bio-assay of a chromatogram of a concentrated aqueous extract of 3 g hull. Two inhibitory zones, repeatedly obtained, can be seen, one at R_f 0.5 to 0.6 and the second broader one at 0.7 to 0.9.

Several experiments have been performed in an attempt to show that these active substances are responsible for the inhibitory effect of the hull.

The hulls from 50 seeds were ground into a powder and mixed with 1 ml distilled water. Fifty caryopses from dormant seeds were then placed on this paste and their germination compared with that of caryopses on moist filter paper. Only small differences were found in the final germination percentages although it was noted that the rate of germination on ground hull was decreased. There was also a reduction in the subsequent root and coleoptile growth in the presence of hull. These results have been obtained in several repetitions. Similarly, when caryopses were placed on a concentrated solution of inhibitors extracted from the hull, germination was not prevented but the rate was reduced. Thus it appears that under these experimental conditions at least, the inhibitory effect of the hull cannot be replaced by supplying inhibitors to the caryopsis, even though these have been obtained from the hull.

A further possible explanation for the ability of isolated caryopses from dormant seed to germinate is that inhibitors are leached from naked caryopses when on moist filter paper, and that this process is normally prevented by the hull.

Water-soluble inhibitors have been found in the caryopsis. Judging from the R_f positions (Fig. 2) these are probably the same as those in the hull, and are present in these tissues in approximately the same concentrations per unit weight.

That these inhibitors are very readily leached out on moist filter paper has been shown in three experiments. One gram of caryopses was placed on wet filter paper for 24 hours. The paper was then macerated in water and stirred for 2 hours at 5° C. After filtration, the filtrate was concentrated and chromatographed. Bio-assay of the chromatogram (Fig. 3) shows the same two inhibitory regions as are present in aqueous extracts of caryopses. There were no inhibitors in the control, an eluate of filter paper.

That leaching is a factor in the germination of isolated caryopses is shown by the following experiments. Fifty caryopses from dormant seeds were placed on moist filter paper and maintained in darkness at 20° C. An equal number were kept under non-leaching conditions in a humid atmosphere obtained by placing several layers of wet filter paper in the top half of a petri dish. Each day it was necessary to remove from around the seeds water which had condensed. After 5 days, the caryopses resting on wet filter paper had germinated 54% compared with only 10% germination in a humid atmosphere. These figures, after 8 days, increased to 62% and 22% respectively, with no further increase during a further 6 days. Similar results have been obtained several times (see, for example, Table II).

Water uptake by the caryopses was essentially the same under the two conditions. Further, it has been repeatedly found that caryopses from *non-dormant* seeds germinate in the same humid atmospheres, at the same rate, and to the same extent as caryopses on wet filter paper. Evidently,

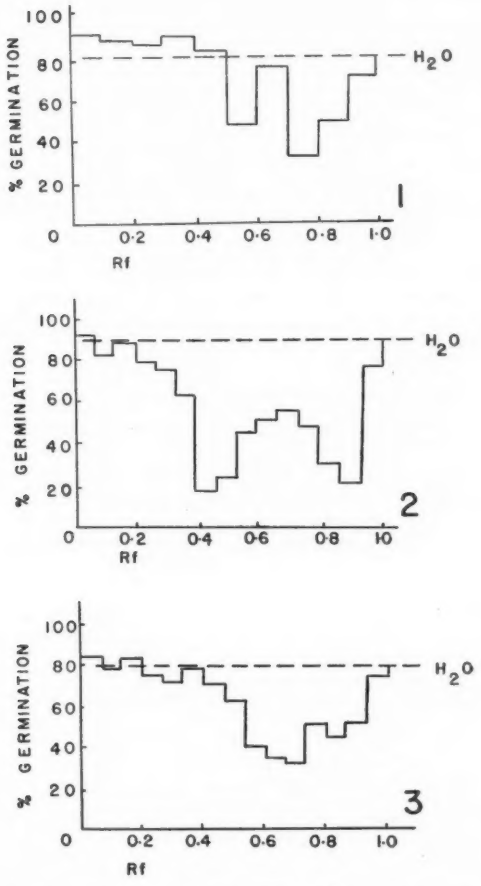

FIG. 1. Bio-assay of a chromatogram of an aqueous extract of hull.
FIG. 2. Bio-assay of an aqueous extract of caryopses (2.5 g).
FIG. 3. Bio-assay of a diffusate from isolated caryopses.

water uptake in a humid atmosphere does not limit germination. It would appear, therefore, that in conditions that preclude leaching, the germination of caryopses from dormant seeds is suppressed; presumably, the leaching of inhibitory substances is the important factor.

The following experiment provides further evidence that germination is promoted under leaching conditions. Dormant seeds were treated in the following ways: (*a*) isolated caryopses were placed on wet filter paper; (*b*) caryopses were maintained in a humid atmosphere as described above; (*c*) the palea and adjacent edges of the lemma were removed, and the seeds were

placed on wet filter paper, with the exposed caryopsis facing upwards, i.e. each caryopsis rested in a boat-shaped portion of the lemma; (*d*) seeds were treated in a similar manner except that they were placed on wet filter paper with the exposed surface of the caryopsis downwards; (*e*) as a control, intact seeds were used. For each treatment three replicates of 25 seeds each were employed. All seeds were maintained in darkness at 20° C and the germination percentages were determined at intervals.

The results in Table II show very marked differences among the effects of these treatments. As expected from previous experiments only a small percentage of caryopses in a humid atmosphere germinated. The difference in response between those seeds in which the caryopsis is in contact with wet paper and those where it is not is very striking and has been obtained repeatedly. These results are completely consistent with the hypothesis that the hull acts by its prevention of leaching.

TABLE II

The effect on germination of leaching conditions

Treatment	Percentage germination			
	1	2	3	Average
Intact	24	36	28	27.7
Caryopsis up	40	40	32	37.3
Caryopsis down	76	88	64	76.0
Isolated, on filter paper	56	68	64	62.6
Isolated, in humid atmosphere	24	24	16	21.3

The prevention by the hull of leaching from the caryopsis has been tested directly in the following experiment. Fifty naked caryopses were allowed to stand on wet filter paper for 24 hours; after this period they were extracted and bio-assayed. Fifty intact seeds were placed on wet paper for the same length of time, after which the caryopses were removed and extracted. A comparison of these bio-assays (Fig. 4) indicates a higher level, especially of one inhibitor, in those caryopses which were imbibed with the hull intact. Further, in the latter the level of inhibitor would appear to be approximately the same as that in dry untreated caryopses, indicating that little inhibitor diffuses inwards from the hull.

The Effect of Oxygen

High oxygen concentrations are known to promote germination of dormant seeds of several species (see e.g. 8). Cutting the seed coat in air is also beneficial, the effect being ascribed to increased oxygen made available to the internal tissues. In this laboratory, we have found that cutting the seed coat, even in the presence of hull, expedites germination of certain "strains" of seed. In another "strain" of seed which is very deeply dormant, germination can be secured only by cutting the seed coat and placing the caryopses in an atmosphere of 100% oxygen. This phenomenon has been investigated with reference to the effect on inhibitor content.

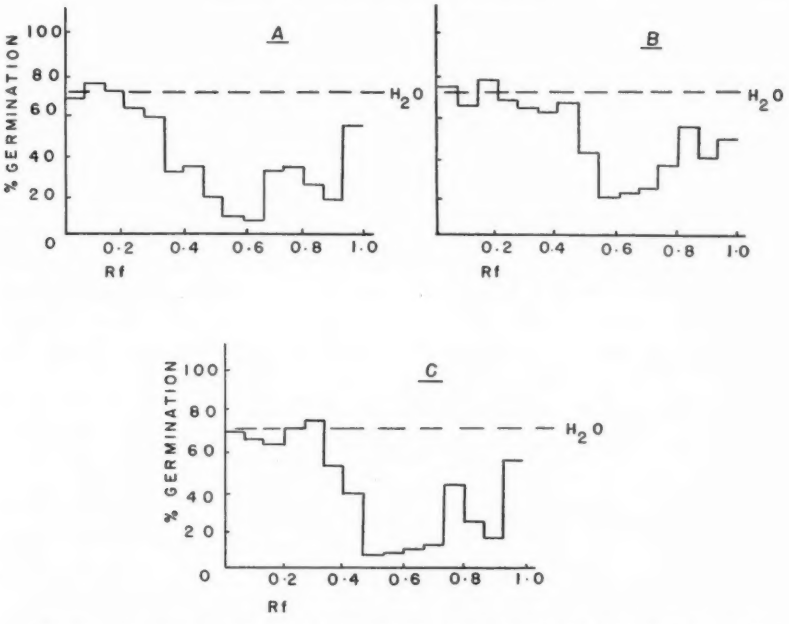

FIG. 4. Bio-assay of extracts of: A, caryopses from intact seeds previously on moist filter paper; B, isolated caryopses previously on moist filter paper; C, dry caryopses.

It has been shown by Wareing and Foda (9) that in *Xanthium* seed there is a marked decrease in inhibitor level on treatment with high oxygen concentrations. Experiments in this laboratory suggest that this is the case for *Avena fatua* seed. Deeply dormant seeds were cut and placed on moist paper in pure oxygen. After 48 hours (i.e. before most of the seeds showed visible signs of germination) they were extracted (with the addition of the filter paper). The extract was then chromatographed and bio-assayed, and compared with an extract of dry seeds (Fig. 5). The decrease in one inhibitor (R_f 0.8–0.9) after oxygen treatment has been confirmed in a repetition of this experiment. Although confirmation for other seed samples would be desirable, it would appear from the results so far obtained that stimulation of germination by high oxygen concentrations can be attributed to the effect of this treatment in reducing the total level of inhibitor in the seed.

An Experiment on Secondary Dormancy

Evidence has been presented above suggesting that naturally occurring inhibitors in the caryopsis are responsible for the maintenance of dormancy. Hence, it might be argued that the intensification or induction of dormancy may be due to increased inhibitor content. In *Avena fatua* dormancy can be induced or intensified in a number of ways, e.g. light, anaerobic conditions, high temperatures (6), or holding dormant seeds in an imbibed condition.

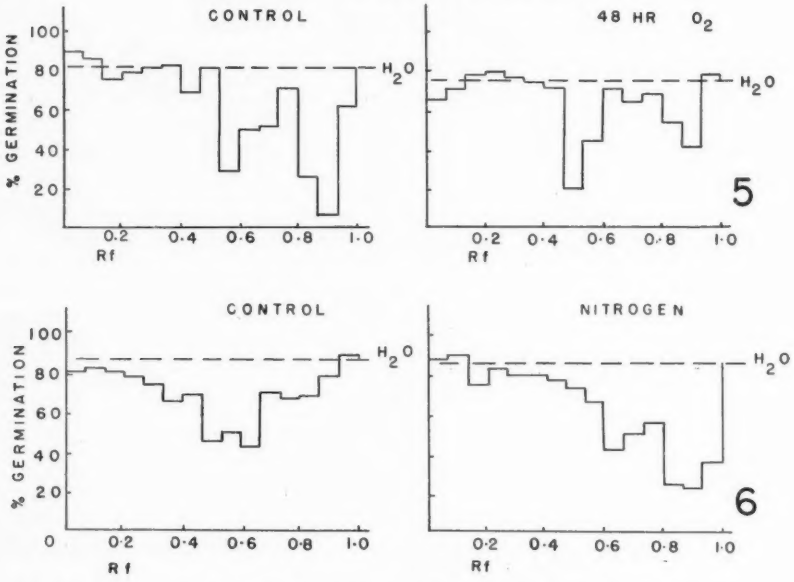

FIG. 5. Bio-assay of extracts of caryopses with and without oxygen treatment.
FIG. 6. Bio-assay of extract of caryopses induced into secondary dormancy with nitrogen.

It has been found that when caryopses from non-dormant seed are held imbibed in a nitrogen atmosphere for 4 days, they will not germinate when afterwards transferred into air, though still alive (3). By use of this treatment, secondarily dormant caryopses have been obtained and investigated with regard to changes in inhibitor content. It has been found in two experiments, using replicates in each case, that there is a marked increase in one inhibitor during nitrogen treatment (Fig. 6), i.e. there is a correlation between the induction of dormancy and the increase of inhibitor. This result provides strong evidence that inhibitors play an important role in dormancy of this seed and shows that the seeds themselves can produce at least one inhibitor.

Discussion

It has been shown above that inhibitors can be extracted from the caryopsis and closely adherent hull of *Avena fatua*.

In analyzing the possible causes of the inhibitory effect of the hull in intact dormant seeds, evidence has been obtained that this effect cannot be attributed solely to its inhibitor content. This is demonstrated by the fact that isolated caryopses from dormant seeds germinate only if they are in contact with free liquid water, although it would appear that the water uptake in a humid atmosphere is adequate to allow of germination (p. 396). This result gives also additional proof that the reduction of gaseous exchange by the hull is probably not a factor involved in the inhibitory effect of this tissue.

The experiment described on page 397 shows that even small portions of the hull are inhibitory but only if these prevent the caryopsis from coming into contact with moist filter paper. Thus, taking these two results into consideration, there is strong evidence that a leaching phenomenon accounts for the ability of isolated caryopses from dormant seed to germinate.

That inhibitors are very readily leached out on moist filter paper has been confirmed a number of times. Moreover, it would appear from a comparison of bio-assays of extracts and diffusates that much of the total extractable inhibitors move out of the caryopsis during 24 hours on moist filter paper.

It is therefore suggested, on the basis of these results, that germination of isolated caryopsis from dormant seed takes place on moist filter paper because of a reduction in the inhibitor level inside the caryopsis. The role of the hull in suppressing germination in intact seeds is thought of as being primarily one of preventing or retarding this loss. Direct evidence for this is given by the results on page 398. The fact that inhibitors very readily diffuse out of the isolated caryopsis might explain our failure to suppress the germination of caryopses placed on ground hull or on paper moistened with inhibitor solution. Such experimental conditions provide also for the outward diffusion of endogenous inhibitor from the caryopsis, and presumably the net effect of this loss and of the entry of exogenous inhibitor is not sufficient to prevent germination.

Thus, the maintenance of dormancy in this seed is held to be due to naturally occurring inhibitors in the caryopsis. Any means of reducing the level of these inhibitors will serve to allow of germination. One method, that of removing the hull so as to provide free leaching, has been considered above. A second method is by increasing the oxygen available to the embryo. This can be achieved by cutting the seed coat in air (for certain strains of seed) or by raising the external oxygen concentration. The effect of cutting the seed coat on inhibitor content has not been investigated. However, it has been shown for a strain of seed which germinates only when cut in 100% oxygen that there is a marked reduction in inhibitor concentration in the seeds after this treatment. This is in agreement with results obtained for *Xanthium* by Wareing and Foda (9).

Further evidence that inhibitors play an important part in the dormancy of this seed is provided by experiments on secondary dormancy. From our results it can be seen that when dormancy is induced under nitrogen there is a marked increase in one inhibitor (R_f 0.8–0.9). Other changes may also occur during induction of secondary dormancy but this possibility has not yet been investigated.

These results demonstrate that the seeds themselves have the capacity for the production of at least one inhibitor. We can understand how several diverse factors such as light, high temperatures, anaerobic conditions, and holding dormant seeds imbibed all serve to induce secondary dormancy if we assume that there are two groups of reactions taking place in the seed, one leading to germination and the second to the formation of inhibitors.

It is conceivable that under favorable conditions the former predominates and the seed therefore germinates. However, if these germination reactions are prevented by, for example, light (reversal of a pigment system as postulated by Borthwick and co-workers (2)) or anaerobic conditions, the inhibitory reaction proceeds and inhibitors accumulate, thus rendering the seed more dormant. The intensification of dormancy when dormant intact seeds are held imbibed would also fit into this idea. In this case, the germination reactions may be suppressed by the already effective level of inhibitor within the seed. If the two groups of reactions have differential temperature coefficients, it is possible that high temperatures favor the inhibitory reactions over the germination reactions, which would explain why secondary dormancy is induced at high temperatures. It is intended to test these speculations in future work.

Since the removal of inhibitors from dormant seeds apparently causes their germination, the question may be raised as to whether there is a loss of inhibitors during the natural afterripening process. From our studies (not reported above) this does not appear to be the case. Although all strains of seeds have not been examined exhaustively, preliminary experiments do not show any differences of inhibitor content between dormant and afterripened seeds. Several other alternative hypotheses can be forwarded to explain afterripening, including the possibility of a specific germination promoter being slowly formed or released from an inactive form. Further studies of the afterripening process, with reference to this particular aspect, are being undertaken.

Nothing is known concerning the chemical nature of the inhibitors in this seed. Chromogenic tests for the common types of germination inhibitors and growth-active substances, such as unsaturated lactones, phenolic acids, and indoles, have yielded negative results.

The author gratefully acknowledges the encouragement and helpful criticism received in discussion with Dr. J. M. Naylor.

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CHROMOSOME BREAKAGE INDUCED IN VICIA FABA ROOT TIPS BY 2,4,6-TRI(ETHYLENEIMINO)-1,3,5-TRIAZINE[1]

RESA WAKONIG[2] AND T. J. ARNASON

Abstract

After a 12-hour treatment in 10^{-5} *M* solution of 2,4,6-tri(ethyleneimino)-1,3,5-triazine (TEM), up to 29% of *Vicia* and 31% of *Allium* root cells in anaphase had bridges or fragments. The most abundant metaphase aberrations were chromosome breaks and chromatid interchanges. Aberrations were more numerous after 6-hour treatment with 2×10^{-5} *M* than after 12-hour treatment with 10^{-5} *M* solution. The peak aberration frequencies were reached at 36 hours after 2×10^{-5} *M* treatments and slightly earlier with weaker solutions. Many chromosomes had incomplete breaks. Sister reunions of chromatids occurred with equal frequency in centric and acentric fragments. Sister reunion was 1.5 times as frequent as chromatid exchange. Along the length of the satellite chromosome, breaks occurred at random except for a slight favoring of the heterochromatic region. Per unit of length the short chromosomes were about 3 times as susceptible to breakage and exchange as satellite chromosomes. TEM introduced through cut stems had no discernible effects on *Tradescantia* microspore chromosomes even after 3 to 5 days of treatment.

Introduction

One of the chemical compounds that has been shown to be mutagenic is 2,4,6-tri(ethyleneimino)-1,3,5-triazine (TEM). This compound was first tested for mutagenic effects after it had been found (2) to inhibit cancerous growths. O. G. and Myrtle Fahmy, in a series of papers (4, 5, 6), reported that TEM is remarkably effective in causing chromosome aberrations and other mutations in *Drosophila*. TEM is also reported to inhibit embryo growth (8).

It would be of interest to determine the effects of TEM on plant chromosomes. Accordingly experiments with three species were carried out and are reported here. Some of the results were summarized at the Tenth International Congress of Genetics (14).

Materials and Methods

Freshly prepared lots of TEM were obtained from the Chester-Beatty Institute through the courtesy of Dr. O. G. Fahmy and Dr. J. L. Everett. The cytological effect of TEM was investigated by analyzing chromosome aberrations after the immersion of plant organs in aqueous solutions of the compound. The material included root tips of *Vicia faba* and *Allium cepa* and microspores of *Tradescantia paludosa*. Since the TEM solutions deteriorated rather rapidly even when kept under refrigeration, the results given are those obtained with fresh solutions of recently manufactured TEM.

The roots of *Vicia* tolerated exposure to 2×10^{-5} *M* solution of TEM for 6 hours and weaker solutions for 12 hours. Temporary mitotic inhibition was observed when roots were treated with 2×10^{-5} *M* and 10^{-5} *M* solutions.

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Contribution from the Biology Department, University of Saskatchewan, Saskatoon, Sask.
[2]National Research Council of Canada Postdoctoral Research Fellow.

Vicia roots, treated for 6 hours with 2×10^{-5} *M* solution and for 6 or 12 hours with 10^{-5} *M* and 10^{-6} *M* solutions of TEM, were fixed at various intervals. *Allium* root tips, similarly treated, were also studied. All treatments were carried out at 20° C, and after treatment the roots were kept in a tank with running tap water maintained also at 20° C. For the observations on *Tradescantia* microspores, stems with bud clusters were immersed in TEM solutions. The buds were fixed at various times up to 6 days after the start of the treatment.

Observations were made on metaphases and anaphases. To facilitate the analysis of metaphases, 0.05% colchicine was used for 2 hours. For root-tip squashes the cells were fixed with Carnoy's mixture (3 parts ethanol:1 part glacial acetic acid). The slides were stained by the Feulgen method. The *Tradescantia* microspores were stained with propionic carmine.

Anaphase Aberrations

The observed frequencies of anaphase aberrations in *Vicia* and *Allium* roots after treatment with TEM are recorded in Table I. A 6-hour treatment of *Vicia* roots with 10^{-6} *M* TEM had no effect on anaphase stages. When the treatment time was extended to 12 hours, aberrations became slightly more numerous than those of control roots grown in tap water. Disturbed anaphases became frequent after exposure to 10^{-5} *M* solutions for 6 hours. When the exposure time was doubled (to 12 hours), the frequency of aberrant cells was also approximately doubled. The highest aberration frequencies appeared at 24 to 36 hours after the end of the treatment.

TABLE I

Frequency of anaphase aberrations in *Vicia* and *Allium* roots after exposure to TEM

Length of treatment, hours	Molar concentration	Hours after treatment	*Vicia*		*Allium*	
			Total cells	% aberrant	Total cells	% aberrant
6	10^{-6}	0–72*	1050	0.6		
12	10^{-6}	0–72*	1050	1.1		
6	10^{-5}	0	150	2.7		
6	10^{-5}	12	150	7.3		
6	10^{-5}	24	140	13.3		
6	10^{-5}	36	150	12.0		
6	10^{-5}	48	150	5.3		
6	10^{-5}	60	150	6.7		
6	10^{-5}	72	90	4.4		
12	10^{-5}	0	150	5.3	29	0
12	10^{-5}	12	150	22.0	100	20
12	10^{-5}	24	150	29.0	100	31
12	10^{-5}	36	150	21.0	100	26
12	10^{-5}	48	150	13.3	100	26
12	10^{-5}	60	150	10.0	—	—
12	10^{-6}	72	150	6.0	100	27
0	—	—†	1900	0.6		

*Seven lots of 150 cells taken at 0, 12, 24, 36, 48, 60, 72 hours.
†Control roots from each seedling taken prior to TEM treatment.

Various authors have suggested that certain chemicals act mainly on heterochromatin. Since *Vicia* has appreciable amounts of heterochromatin but *Allium* does not, it is of interest to compare the effects of TEM on the chromosomes of these species.

The aberrations observed in both species were bridges and fragments. No chromosome stickiness occurred and very few pseudochiasmata were seen. The frequencies of anaphase aberrations (Table I) and the types of metaphase aberrations were very similar in *Allium* and *Vicia* after the treatments with TEM at 10^{-5} *M* for 12 hours. However, a somewhat higher proportion of minute fragments occurred in *Allium* than in *Vicia*. One hundred aberrant cells of *Allium* contained 46 minutes, while in 100 aberrant cells of *Vicia* only 15 minutes were found. In both species micronuclei were present in many cells at about 36 hours after the end of treatment. Since the results were similar for the most part in the two species, it may be concluded that TEM affects chromosomes regardless of whether heterochromatin is present or not.

Metaphase Aberrations

Detailed information about the nature of the TEM-induced chromosome changes was obtained by a study of metaphases. The frequency of aberrant metaphases was low immediately after the conclusion of 6- or 12-hour treatment with 10^{-5} *M* TEM. After recovery for 24 to 36 hours in water, aberrations became frequent. This is illustrated in Fig. 1. From 48 hours to 84 hours, the aberration frequency dropped rapidly. Few metaphase breaks were seen after a 12-hour treatment with 5×10^{-6} *M* TEM. At concentrations

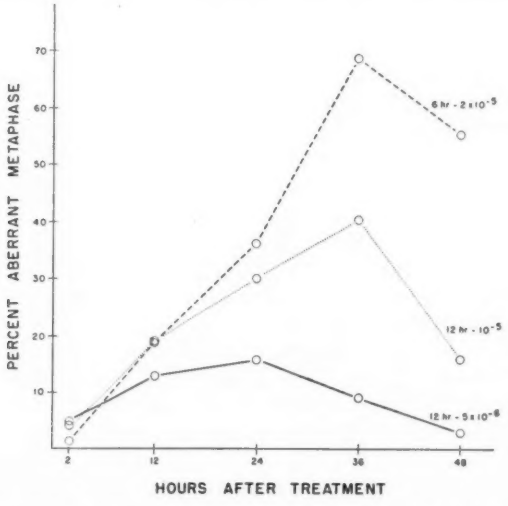

FIG. 1. Frequency of aberrant metaphase cells in *Vicia* root tips fixed at various times after the end of TEM treatments.

of 10^{-5} *M* for 12 hours and with 2×10^{-5} *M* for 6 hours, aberrations were common. The frequencies of different kinds of aberrations are listed in Table II.

TABLE II

Frequency of chromosome aberrations in *Vicia faba* root tips after exposure to TEM (all recovery periods pooled)

Molar concentration	Metaphases analyzed	Aberrations per 100 cells						
			Chromatid interchanges			Chromatid intrachanges	Chromatid breaks	Minutes
		Isolocus breaks	Triradials	Quadriradials	Others			
5×10^{-6}, 12 hours	1078	4.8	—	0.3	—	—	1.5	—
10^{-5}, 12 hours	1170	17.3	0.9	7.5	1.2	1.1	2.7	2.3
2×10^{-5}, 6 hours	491	25.9	2.2	16.9	1.8	4.7	6.1	8.0

In classifying open breaks we found that chromosome (isolocus) breaks were much more numerous than were chromatid breaks (see Table III). The reunions were nearly all of the chromatid type. Dicentric chromosomes were not seen and very few ring fragments were found up to 48 hours. After 72 hours some dicentrics appeared, but these were in cells undergoing second or later mitoses. It is not easy to see why exchanges should be almost all chromatid and almost none chromosome exchanges. The chromosomes must be effectively split before exchanges occur. Exchange rather than sister reunion might be favored if at a breakage locus the two chromatids do not complete the breaks simultaneously. That this may happen is indicated by our observations on incomplete breaks. In some of these one chromatid was broken; the other chromatid still held together by a fine thread at the breakage locus.

Chromatid exchanges recorded in Table III were more numerous after a 6-hour treatment with 2×10^{-5} *M* solution of TEM than after a 12-hour treatment with 10^{-5} *M* solution. The peak frequency was reached somewhat later with the stronger than with the weaker solutions. Most of the exchanges were identified in quadriradial configurations. That these effects are not attributable to colchicine was shown by checking control root tips treated for 2 hours in colchicine and then fixed. In 100 cells so treated no exchanges and only one fragment was found. This is quite similar to the results obtained with control root tips grown in tap water. In 1900 untreated cells, 11 (0.58%) had fragments; none had exchanges.

In addition to completed breaks, incomplete or partial breaks were observed at metaphase. The appearance of these ranged from a slight bilateral constriction or indentation to a mere thread connection between fragments. The highest frequency of partial breaks observed was 75 in 100 cells (after treatment with 2×10^{-5} *M* TEM). These incomplete breaks may be completed or restituted (1) later. If in a partial break not all the strands of a chromatid are broken, segregation of these may occur at later divisions (6). The completion of breakage at later divisions may result in mosaic conditions

such as have been observed in TEM-treated *Drosophila*. Mosaics in *Drosophila* are common after some other chemical treatments (1). Whether the lesions represent potential breaks (see Östergren and Wakonig (12) for discussion) or stages in restitution, they must be structurally weak and, hence, more likely than other parts of chromosomes to break.

TABLE III

Frequency of chromosome aberrations in *Vicia faba* root tips fixed at various times after exposure to TEM

Molar concentration	Hours after treatment	Metaphases analyzed	Aberrations per 100 cells			
			Isolocus complete	Breaks incomplete	Chromatid breaks	Chromatid exchanges
5×10^{-6}	2	261	3.8	6.1	1.9	0
5×10^{-6}	12	130	3.9	11.5	0.7	2.3
5×10^{-6}	24	203	10.9	10.2	3.5	3.0
5×10^{-6}	36	209	2.8	5.8	1.0	0.5
5×10^{-6}	48	275	3.3	4.4	0.4	0
10^{-5}	2	234	3.4	7.3	4.3	.9
10^{-5}	12	146	11.0	20.5	4.1	6.9
10^{-5}	24	133	18.8	20.3	3.8	24.2
10^{-5}	36	330	24.2	24.5	2.4	20.3
10^{-5}	48	327	22.3	7.7	0.6	1.5
2×10^{-5}	2	122	0.8	3.3	0.8	0
2×10^{-5}	12	94	7.5	14.9	2.1	4.3
2×10^{-5}	24	105	42.9	56.2	9.5	21.9
2×10^{-5}	36	67	58.2	74.6	19.4	53.7
2×10^{-5}	48	103	34.0	29.2	3.8	41.8

Fragments were examined for the occurrence of sister reunion. When fragments from all fixation times were totalled (Table IV), it was found that more than one-half of each of the centric and acentric fragments had achieved complete reunion. This is in contrast to the conditions found after mustard gas treatments (7). Weak or incomplete reunion occurred in less than one-tenth of the fragments, the remainder showing non-reunion. That the sister reunions were often weak was shown by the frequent breakage of anaphase bridges at the mid-point.

TABLE IV

Frequency of sister reunions (SR) in *Vicia faba* roots after exposure to 10^{-5} *M* TEM for 12 hours

Hours after treatment	Metaphases analyzed	Fragments per 100 cells					
		Centric fragments		Acentric fragments		Total	
		SR	Non SR	SR	Non SR	SR	Non SR
2	234	1.3	2.1	1.3	1.7	2.6	3.8
12	146	6.8	4.8	4.1	5.5	11.0	10.3
24	133	12.8	13.5	16.5	9.8	29.3	23.3
36	330	13.0	5.5	13.0	5.5	26.1	10.9
48	327	5.2	6.4	4.6	5.5	9.8	11.9
Total	1170	7.7	5.9	7.6	5.2	15.3	11.1

The relative frequencies of sister reunions and chromatid exchanges were determined in the material treated for 12 hours with 10^{-5} *M* TEM. When the results from all fixation times were pooled, the ratio SR:translocation was 1.5:1. At 24 and 36 hours the ratio was close to 1:1 with greater divergence at earlier and later fixation times.

The Distribution of Breakage Points in the Satellite Chromosome of TEM-treated *Vicia*

Several regions of the satellite chromosome of *Vicia* can be identified cytologically. These regions are indicated in the diagram in Fig. 2. The main heterochromatic blocks are near the centromere (9, 10), i.e. the short regions labelled B and C in the figure. Several authors (7, 10, 13) have found that after treatment with chemicals heterochromatic regions are sites of preferential breakage. Breaks in the centromere constriction and in the achromatic region between the satellite and the short arm were not scored. It is known that some breaks occurred in the second of these regions since the chromosome at the next mitosis occasionally lacked the satellite. In our figures the recorded breakage in the arm including the C, D, E sections is somewhat less than the actual total breakage for the whole arm since some breaks occur in the achromatic part.

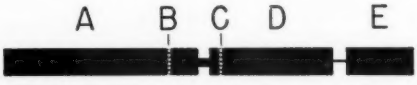

FIG. 2. Labelled diagram to represent the satellite chromosome of *Vicia*. The constriction between B and C is the centromere; E is the satellite. The heterochromatin is in segments B and C.

Breakage points were determined from satellite chromosomes with open breaks and those involved in quadriradial configurations. All five of the labelled regions had some breaks (Table V). The distal part of the long arm (region A in Fig. 2 and Table V) had between 23 and 50% of the breaks occurring in the chromosome. If breaks occur at random, this is about the

TABLE V

Distribution of breaks in the satellite chromosomes of *Vicia faba* roots exposed to TEM. Chromosome segments labelled as in Fig. 2

Concentration TEM	Total number	A	B	C	D	E
I. Breakage frequency (%) in labelled segments						
5×10^{-6} *M*	10	50	0	30	10	10
10^{-5} *M*	74	44.6	13.4	25.7	8.1	8.1
2×10^{-5} *M*	46	32.6	10.8	28.3	8.7	19.6
II. Frequency (%) of incomplete breaks in segments						
5×10^{-6} *M*	24	4.2	54.2	41.7	0	0
10^{-5} *M*	86	23.3	26.7	32.6	4.6	12.8
2×10^{-5} *M*	32	25.0	37.5	28.2	3.1	6.3

proportion expected. The short proximal parts B and C had rather more, and region D rather less, than their fair share of breaks. It appears, therefore, that breakage is more likely to occur in heterochromatic than in euchromatic regions of equal length. This is no more than a tendency, however, since many breaks occur in euchromatic parts.

The incomplete breaks in the satellite chromosome, like the complete breaks, occurred in all five of the labelled regions (Table V, Part II). These were, however, relatively much more frequent in the parts that include the heterochromatin blocks (B and C) than elsewhere, especially after treatment with a very weak concentration of TEM. If these are weak spots, perhaps the distribution of breaks opening up at later divisions will be somewhat different from that occurring immediately after the treatment.

Observations were also made on the other chromosomes involved in exchanges. The satellite chromosome most often had a short chromosome for its exchange partner. Short chromosomes also exchanged with each other. The frequencies of the different kinds of exchanges are listed in Table VI. If chromosome breaks were proportional to chromosome length, the total breakage in the short chromosomes should be 2.2 times the number in the satellite chromosomes (7). On this basis short chromosomes take part in translocation more frequently than would be expected if the distribution were random. These were represented in exchanges 5.7 times as often as the satellite chromosomes. This, as well as Ockey's data (11), indicates that some or all of the short chromosomes are more susceptible than the satellite chromosomes to breakage and exchange after TEM treatment.

TABLE VI

The frequencies of different chromosome combinations in exchange configurations after TEM treatment

Molar concentration TEM	No. of metaphases	Combinations (%)			Chromosome ratio, Sat.: short
		Sat.–sat.	Sat.–short	Short–short	
5×10^{-6}	1078	0	0.3	0.6	1:5.7
10^{-5}	1170	0.3	2.4	7.3	1:5.8
2×10^{-5}	491	0.6	5.5	15.9	1:5.6

The Effects of TEM on Chromosomes of *Tradescantia* Microspores

Cut stems of *Tradescantia* were immersed in TEM solutions for 1 to 5 days to determine whether effects on microspore divisions could be produced. After 2 days in 10^{-3} *M* solution of TEM and 2 days recovery in water, visible effects on microspore divisions were negligible (1 break in 100 cells). Very few divisions occurred immediately after treatment for 24, 48, or 72 hours. The chromosomes in the dividing cells were all intact in about 99% of cells. Either very little TEM gets into the microspores or else the nuclei had already passed the sensitive stages when the treatment was begun. Some dividing cells observed at 5 days after the beginning of treatment still showed no chromosome aberrations.

Discussion and Conclusions

Our results and those of Fahmy (4, 5, 6) and Ockey (11) agree in finding that TEM at certain concentrations is capable of producing numerous chromosome breaks. The virtual absence of chromosome rearrangement, while chromatid exchanges are common, provides an intriguing puzzle. McLeish (10) obtained rather similar reunion results in *Vicia* using maleic hydrazide. His explanation is that, when sister reunion fails to occur at the time of the chromosome split, the free ends can then take part in chromatid reunions.

If the first effect of the chemical is to produce a potential but not an actual break in the chromosome, the completion of breakage in sister chromatids might not occur at the same instant. Single chromatid breaks might then be open for reunion with similar breaks in non-sister chromatids. Following such reunions the remaining chromatids might, if their potential breaks become actual, have no alternatives but to restitute or to remain open. Chromatids were sometimes constricted at points opposite to the break exchange loci of their sister chromatids, but they rarely had open breaks in these positions.

The points along chromatids at which exchanges occurred were many, the distribution being essentially random. Individual chromatids exchanged, in different cells at many points with various other chromatids. Our observations, therefore, give no confirmation for the opinion (3, 10) that the anchoring of chromosomes in resting nuclei by blocks of heterochromatin and by the nucleoli will limit chromosome contacts and hence exchanges to particular regions.

Of the many TEM-induced exchanges involving the satellite chromosome, all but two certainly had non-homologous exchange points. The partner for satellite-chromosome exchange was usually one of the short chromosomes. Exchanges between non-homologous short chromosomes were also observed. This is in contrast to Revell's (13) observations after treatments with diepoxide. He reported that a considerable fraction of the reunions linked homologous chromosomes at apparently homologous points. He inferred that unusual pairing of homologues with chiasma formation as in meiosis might be occurring.

Open chromatid breaks were few in our material, but these were more frequent at some fixation times than others. The frequency of both chromosome and chromatid breaks is affected by the concentration of the compound and by the length of the exposure period. Ockey (11) found that after treatment for 1 hour in TEM solutions chromatid breaks were nearly equal to chromosome breaks in frequency. In our material, treated for 12 hours, the chromatid breaks were always much less numerous than the chromosome breaks.

The effectiveness of TEM in inhibiting growth of tumors and embryos probably results in part at least from the serious damage inflicted on chromosomes. The breakages, complete and potential, accompanied by sister

reunions and chromatid exchanges must often prove cell-lethal. In genetic work TEM might be useful as an agent to induce rearrangement of chromosome segments.

Acknowledgments

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CYTOGENETIC STUDIES IN AMARANTHUS

I. CYTOLOGICAL ASPECTS OF SEX DETERMINATION IN DIOECIOUS SPECIES[1]

WILLIAM F. GRANT

Abstract

Cytological observations are contained in this report on four dioecious species of *Amaranthus*, two of which were formerly included in the genus *Acnida*. The species studied are *A. arenicola*, *A. palmeri*, *A. tamariscinus*, and *A. tuberculatus*. Three of these species have a diploid chromosome number of 32, whereas *A. palmeri* has a somatic chromosome number of 34. A single spontaneous triploid ($2n = 48$) female plant was found in collections of *A. tamariscinus* and a tetraploid ($2n = 64$) male plant in collections of *A. tuberculatus*. A fifth species, *A. australis*, has previously been reported as having 32 somatic chromosomes. The chromosome numbers for half the dioecious species of *Amaranthus* have now been determined. Detailed observations on the dividing chromosomes of these species in mitosis, in meiosis, and in the first division of the nucleus in the pollen grain have been made and have failed to distinguish heteromorphic chromosomes which might be associated with sex determination. The small size of the chromosomes has made detailed morphological studies impractical and there is no marked difference in absolute size of the chromosomes between species. Since haploid numbers of 16 and 17 are found in both monoecious and dioecious species, it would seem that the aneuploid condition in *Amaranthus* arose early and hybridization within the genus has resulted in promoting the genic condition which has been necessary for the expression of the dioecious condition.

Introduction

Amaranthus is a tropical cosmopolitan genus comprising some 50 or more species (8, 19) a few of which are world wide in distribution (5). The amaranths are perhaps best known as successful weeds; however, in tropical and subtropical countries, certain species are grown for grain (12), whereas the young plants of other species are eaten for vegetable greens (2). In addition, some members are cultivated for their highly colored inflorescences.

Unlike the monoecious species which are widely distributed, the dioecious species are found entirely on the North American continent. A monograph of these species has recently been completed by Sauer (13), who has also plotted their geographical ranges from their herbarium record (14). Of the ten species which comprise this group, four species formerly belonged to the genus *Acnida*, and one species to the genus *Acanthochiton*.

The study of these four dioecious species of *Amaranthus* was undertaken to determine their chromosome numbers and karyotypes. This information is of importance for the following reasons:

(1) As evidence for including all four species in *Amaranthus*, or placing two of them in *Acnida* as has been done by some taxonomists.

(2) To see if the karyotype can be correlated with sex determination. From genetic data, the male plant has been shown to be the heterogametic

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sex in *Amaranthus*, and it has been postulated that there might be *X* and *Y* chromosomes for sex determination (10). Since sex chromosomes have been reported in a number of different plant groups and have been useful for certain types of cytogenetic studies (18), this information might be of further interest in *Amaranthus*.

(3) To compare the chromosome numbers of the four dioecious species with those numbers obtained by the author and others (4), for various monoecious species.

Materials and Methods

Specimens were grown for cytological study from seed collected from plants growing in their natural habitats. A few accessions were the result of obtaining viable seed from recent herbarium specimens. Mitotic chromosomes of both male and female plants were studied from root tips of potted plants. Root tips were pretreated to facilitate the determination of chromosome number by placing them in a saturated solution of monobromonaphthalene (11) within a refrigerator at 10° C. The desired contraction of the chromosomes was obtained in 30 minutes to 1 hour. Root tips were fixed in CRAF, processed by the Feulgen method (3), embedded, sectioned at 8 μ, and stained according to Newton's crystal violet – chromic acid schedule (3). This double staining procedure of Feulgen and crystal violet resulted in brilliantly stained chromosomes with a clear background. Root tips were also fixed without pretreatment and stained in leuco-basic fuchsin (Feulgen) alone for observations of chromosome morphology. Meiotic chromosome studies were made on anthers squashed in 45% acetic acid or iron aceto-carmine, after the flower buds had been fixed in acetic–alcohol (1:3) and

TABLE I

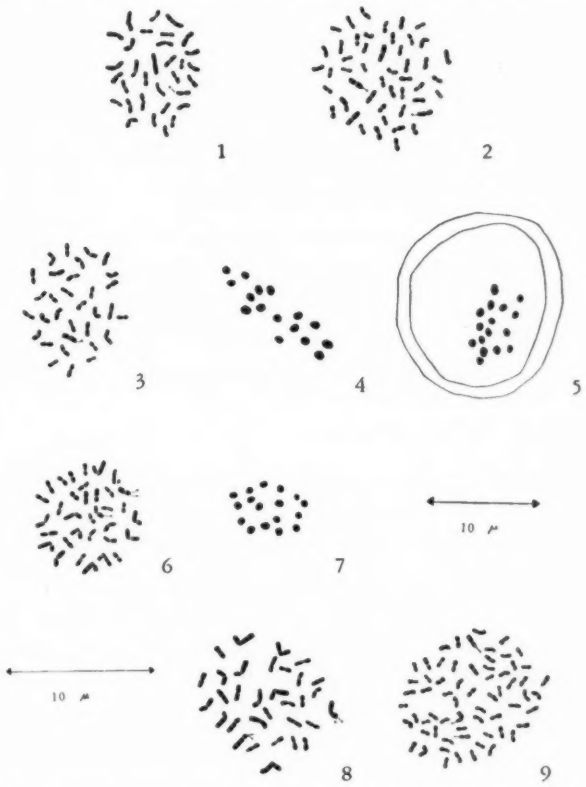
Chromosome numbers of dioecious species of *Amaranthus* and sources of material

Species	Acc. No.	Source	2*n*	Authority
Amaranthus arenicola I. M. Johnston	265	Roadside, Merriman, Nebraska. Collector, J. Sauer, No. 1726	32	Grant
	292	Experimental Range, U. S. Southern Great Plains Field Station, Fort Supply, Oklahoma. Collector, D. H. Gates	32	Grant
A. australis (A. Gray) J. Sauer		Listed as *Acnida cuspidata* Bert.	32	Murray (10)
A. palmeri S. Wats.	278	Campus, University of Arizona, Tucson, Pima Co., Arizona. Collector, C. T. Mason, Jr.	34	Grant
	279	Grass nursery, College Station, Texas. Collector, R. P. Celarier	34	Grant
	280	Farm, Sutherland Springs, Texas. Collector, R. P. Celarier	34	Grant
	281	Urvaldia, Texas. Collector, R. P. Celarier	34	Grant
	286	Five miles S. George West, Live Oak Co., Texas. Collector, L. H. Shinners	34	Grant
	299	Brownsville, Texas. Collector, Robert Runyon	34	Grant
A. tamariscinus Nutt.	270	North of Johnston, Iowa. Collector, Edgar Anderson	32, 48	Grant
		Listed as *Acnida tamariscinus* (Nutt.) Wood	32	Murray (10)
A. tuberculatus (Moq.) J. Sauer	266	Cornfield, Lake Mendota, Madison, Wisconsin. Collector, J. Sauer, No. 1727	32	Grant
	271	East Peoria, Illinois. Collector, V. H. Chase, No. 10900	32	Grant
	298	South Amana, Iowa. Collector, W. Easterly, No. 1252	32, 64	Grant
	316	Sec. 10. Medina Twp., Illinois. Collector, V. H. Chase, No. 13094	32	Grant
		Listed as *Acnida tuberculata* Moq.	32	Murray (10)

stained by the Feulgen method (3). Herbarium specimens of the different collections are deposited in the herbaria of the Botany and Plant Pathology Division, Department of Agriculture, Ottawa; the Smithsonian Institution, Washington; and the University of Wisconsin. I am indebted to Dr. J. Sauer at the latter institution for verifying the identification of the specimens.

Observations

The different species which have been examined are given in Table I along with their chromosome numbers and their source. Camera lucida drawings of the chromosomes from root tips which were fixed without pretreatment for each species are shown in Figs. 1 to 9. The drawings are all from male



FIGS. 1–9. Somatic metaphase chromosomes of dioecious species of *Amaranthus*, except Figs. 4 and 7 meiotic metaphase and Fig. 5 metaphase complement in pollen grain. Magnification × ca. 3850, except Figs. 4, 5, and 7 × ca. 2933. Figs. 1 and 9. *Amaranthus tuberculatus* (Moq.) Sauer. Fig. 1. Diploid, $2n = 32$. Fig. 9. Tetraploid, $2n = 64$. Figs. 2–5. *A. tamariscinus* Nutt. Fig. 2. Triploid, $2n = 48$. Fig. 3. Diploid, $2n = 32$. Fig. 4. Metaphase I, $n = 16$. Fig. 5. Mitotic metaphase, $n = 16$. Figs. 6–7. *A. palmeri* S. Wats. Fig. 6. $2n = 34$. Fig. 7. Metaphase I, $n = 17$. Fig. 8. *A. arenicola* I. M. Johnston, $2n = 32$.

plants with the exception of Fig. 2 which represents the chromosomes of a triploid female plant of *A. tamariscinus*. In addition to this triploid, which was found in an examination of the chromosomes of a large number of individual male and female plants, a tetraploid male plant of *A. tuberculatus* (Fig. 9) was also found. All species have a pair of chromosomes bearing satellites, and this feature has been noted in monoecious species as well (unpublished observations). The mitotic chromosomes of both male and female plants of the same species could not be differentiated on the basis of morphology. The chromosomes are relatively uniform in size, and coupled with their over-all small size (2–3 μ in length) and relatively large number are not particularly favorable for cytological study.

Meiotic chromosomes were studied in prophase through first anaphase and no heteropycnotic chromosomes were observed nor were any morphological features which would distinguish the chromosomes. It was found that the first division of the nucleus in the pollen grain occurred before the pollen was shed (Fig. 5), but no morphological differences were observed between the chromosomes at this stage.

Discussion

Although the small size of the chromosomes in *Amaranthus* might contribute to the inability to detect a small morphological variation, it is considered that heteromorphic chromosomes associated with sex are absent. In plants where such chromosomes have been definitely established, they may be readily distinguished from the autosomes (9, 15, 17). Since the dioecious condition in *Amaranthus* is considered relatively recent (14), as it is for many other plants (18), differentiated sex chromosomes are most likely a secondary development and therefore their presence should not be expected in all plants possessing the dioecious habit.

As may be seen from Table I, somatic chromosome numbers of both 32 and 34 are represented in the dioecious species. These numbers are also found for the monoecious species of the genus (4 and unpublished observations). That there is no difference in chromosome number between the monoecious and dioecious species is not surprising as it has been shown that sex expression can be changed through the action of one or more genes (18). The best known example in which the dioecious condition has been produced artificially has been in the production of dioecious strains of maize which have resulted through the interaction of two genes (7). A number of diploid species have been shown to evolve gradually from some particular ancestor and in so doing change their haploid chromosome number (16). Since haploid numbers of both 16 and 17 are found in both monoecious and dioecious species, it would seem that the aneuploid condition arose early in *Amaranthus*, while the dioecious habit has been a secondary phenomenon arising in the American species. Since natural interspecific hybridization is known to be abundant within the genus (14), the dioecious condition has most likely arisen as a result of hybridization which has brought together the required

genic condition for its expression. This, however, cannot rule out direct mutation.

In some genera, a study of the karyology of its different members has contributed significantly to an understanding of the evolutionary trends within the genus (16). The small size of the chromosomes in *Amaranthus* does not lend them to a detailed study. The chromosomes, however, seem to be characterized by largely median or submedian centromeres. Centromere position of this kind has been associated with primitiveness in some genera (e.g. *Crepis* (1)) but no correlation between centromere position and evolutionary trend seems feasible in *Amaranthus* since there is very little variation between the karyotypes of the different species.

The fact that the chromosome numbers of the species which were formerly classified in the genus *Acnida* are the same as those found in *Amaranthus* lends support for their inclusion in the latter genus.

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SPECIES OF LEPTOTHYRIUM AND KABATIA ON LONICERA[1]

I. L. CONNERS

Abstract

A comparative study of specimens in several herbaria has revealed that the species of *Leptothyrium* and *Kabatia* already reported on *Lonicera* fall naturally into several well-marked varieties, each of which is confined to one or a few hosts. Three new combinations, *Leptothyrium periclymeni* var. *xylostei*, *Kabatia lonicerae* var. *americana*, and *K. lonicerae* var. *latemarensis*, are made and two new varieties, *K. lonicerae* var. *involucratae* and *K. mirabilis* var. *oblongifoliae*, are described. *Leptothyrium periclymeni* attacks a limited number of hosts, which, however, occur in both subgenera of *Lonicera* whereas the *Kabatia* species parasitize hosts belonging to a section of one subgenus.

Introduction

The fungi that will be treated in this paper are parasitic on the leaves of several species of *Lonicera*. As Diedicke (4) and Müller (9) have already stressed, they are relatively common on their respective hosts, although they are rather poorly represented in most herbaria. Indeed, on occasion they may cause considerable damage to the foliage as attested by Müller (9) for *Kabatia lonicerae* on *Lonicera coerulea* in Grächen, Switzerland, and confirmed by my own observations on *K. mirabilis* on *L. oblongifolia* near Ottawa, Ontario. The various species of *Lonicera* grow in woods, along banks of streams, on swampy ground, or in mountainous terrain but rarely occur in any great concentration. Consequently, although these fungi cause a conspicuous leaf spot, a considerable area usually must be traversed to make a sizeable collection in any one locality.

These fungi cause circular, often somewhat irregular, spots, conspicuous from the upper surface, 2–10 mm in diameter, frequently confluent, and occasionally involving larger areas, at first either greenish gray with a broad brown border, or light tan or ashen with reddish-brown border, often translucent, and finally with the central area partly eroded away. The pycnidia are discoid, epiphyllous, rarely also on the lower surface, shining black (yellowish buff in *Kabatia mirabilis*), scattered or irregularly grouped in the central portion of the spot, occasionally confluent, few to several in a spot or even numerous in large ones, 110–200 μ in diameter, apparently subcuticular, with a plane or slightly concave hymenial layer below and with a single layer above of convergent, septate, more or less irregular, laterally fused hyphae arising at the periphery of the pycnidium, opening irregularly by one or more clefts in the covering layer and finally exposing most of the hymenium. Spores continuous or one-septate, inequilateral, falcate, occasionally straight, and in *Kabatia* often elongate, the lower cell enlarged toward the base and the upper attenuate toward the apex.

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Particular attention has been paid to the attachment of the spores because of continued lack of agreement among students of the group. Bubák and Kabát (2) appear to have been the first to observe the attachment correctly, but Overholts (10) quite independently came to the same conclusion. My own observations confirm their findings that the spores in *Kabatia* are attached by their broad basal cell and narrowed above into a nearly straight peg-like or elongate cell. If the spores are examined under high magnification a point will be found where the gently curving wall is perceptibly flattened. When the spores were viewed in the dark field of a phase-contrast microscope, the attachment scar was unmistakable. Occasionally when the upper cell was very long, a second septum was present. Very rarely a forked apical cell has been seen. The attachment of the spores was probably misinterpreted because the septum of the spore is very thin and may not be detected unless the spore occupies a position precisely parallel to the plane of the slide. Small and presumably young spores are continuous and the conidiophores to which they are attached may be mistaken for the apical cell. In freehand sections of the affected leaf tissue a few spores with the septum visible were found still attached to their conidiophores. In these sections and in mounts of more or less complete pycnidia mounted in face view and gently crushed, the spores are oriented with the attenuated apices converging toward the center of the pycnidium. In *Kabatia lonicerae* var. *involucratae* of which the spores are large and the apical cells relatively large, no other spatial relation of the spores within the enclosing pycnidium seems possible.

In his treatment of the Leptostromataceae in 1914, Diedicke (4) noted that the fruit bodies of these fungi on *Lonicera* were similar to those in genera of the Melanconiaceae except that the acervulus-like hymenium is enclosed by a one-celled layer of elongated hyphae. Shortly afterwards von Höhnel (6) erected *Colletotrichella* as a new genus of the Melanconiaceae based on *Labrella periclymeni* Desm. and regarded *Kabatia* as a closely related genus. These authors are probably correct in their interpretation, but until a more exhaustive treatment of these forms is available I propose to retain these species in *Leptothyrium* and *Kabatia*. Eventually they might more properly be considered members of the same genus containing species with continuous and one-septate spores.

Recently Müller (9) described *Guignardia latemarensis* from dead leaves of *Lonicera coerulea* collected in the spring in Switzerland. Cultures of the ascospores on malt agar yielded spores of a *Kabatia* identified as those of *K. lonicerae* (*K. latemarensis*) on this host. Apparently no attempts were made to confirm the connection between the two fungi by making par allel cultures on malt agar of field-collected spores of *K. latemarensis* or by infection experiments on the host. He finds that the *Guignardia* fruits in early spring and only the empty perithecia are found on overwintered leaves later in the season. As Müller notes, his fungus differs morphologically only slightly from *G. lonicerae* Dearn. (3) on *Lonicera hispidula* Dudl., but it should be emphasized at once that physiologically the two fungi are quite different as

G. lonicerae occurs in necrotic spots on the living leaves of the host. Moreover, as far as I am aware, no conidial fungus belonging to the group being discussed in this paper has been found on *L. hispidula*.

Taxonomy

Our present knowledge of the fungi here reported begins in 1848, when Desmazières described *Labrella periclymeni* on *Lonicera periclymenum*. It was possible to see the types of those species the identity of which was critical to the taxonomy of the group. Types of the facultative synonyms were not seen, but from the published descriptions and figures it was possible to be reasonably certain of their correct disposition.

The key to the species was drawn up primarily as an aid to their identification, but it also gives a fairly natural arrangement of the individual species and varieties. It is probable that the coloring of the pycnidia at maturity is more fundamental than the septation of the spores. Spore size is more variable and clearly secondary in importance.

The following code letters are used in referring to herbaria in which the specimens cited are deposited: BPI—Division of Mycology and Disease Survey, U.S. Department of Agriculture; CUP—Department of Plant Pathology, Cornell University, Ithaca; DAO—Phanerogamic Herbarium, and DAOM—Mycological Herbarium, Botany and Plant Pathology Division, Science Service, Ottawa; IMI—Commonwealth Mycological Institute; KP—Plant Pathology Laboratory, Kentville, N.S.; NY—New York Botanical Garden; TRT—University of Toronto Phanerogamic Herbarium, and TRTC—University of Toronto Cryptogamic Herbarium; UC—University of California, Berkeley; WIS—University of Wisconsin, Madison. Slides prepared from these specimens with few exceptions are deposited in the herbarium at Ottawa.

Key to the Species

1. Spores continuous.
   2. Spores usually less than 26 μ in length and less than 9 μ in width..... *Leptothyrium periclymeni* var. *periclymeni*
   2. Spores stouter, up to 34 μ in length and up to 12 μ in width..... *L. periclymeni* var. *xylostei*
1. Spores two-celled.
   3. Pycnidia black at maturity.
      4. Spores often quite short, 14.5–30×5–10 μ; apical cell 4–15.5 μ, basal cell 14.5–19.5 μ..... *Kabatia lonicerae* var. *americana*
      4. Spores longer, 27–32×5.5–8.5 μ; apical cell 7–14.5 μ, basal cell 13–20 μ..... *K. lonicerae* var. *lonicerae*
      4. Spores still longer, 27–34.5×5.5–8.5 μ; apical cell 7–21.5 μ, basal cell 16–22 μ..... *K. lonicerae* var. *latemarensis*
      4. Spores very large, 33–47×5.5–11.5 μ; apical cell 7.5–24 μ, basal cell 24–42.5 μ..... *K. lonicerae* var. *involucratae*
   3. Pycnidia buff at maturity.
      5. Spores medium length, 19–33×7–10 μ; apical cell 3–6 μ, basal cell 16–27 μ..... *K. mirabilis* var. *oblongifoliae*
      5. Spores longer, 21.5–45.5×6.5–13 μ; apical cell 11.5–21.5 μ, basal cell 17–27 μ..... *K. mirabilis* var. *mirabilis*

LEPTOTHYRIUM PERICLYMENI (Desm.) Sacc., Syll. Fung. **3**, 626 (1884), var. PERICLYMENI.

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Colletotrichella periclymeni (Desm.) Höhn., Sitzungsber. Kais. Akad. Wiss. Wien, **125**, 100 (1916).

Spores continuous, straight or broadly falcate, 14.5–25.5 (29.5) × 4.5–8.5 μ.

Type.—On *Lonicera periclymenum* L.; Desm., Pl. Crypt. Fr. *76* [sér. II. 1853].

Specimens Examined

On *Lonicera caprifolium* L. EUROPE: **Yugoslavia**: Ljubjana (as Carniola: Laibach), Thuem., Mycoth. univ. *1893*, ex BPI (as *L. pictum*). **Hungary**: prope Szentgyörg, Krypt. Exs. ed. Mus. Palat. Vindebon. *835*, ex BPI.

On *L. periclymenum* L. EUROPE: **France**: no locality, Desm., Pl. Crypt. Fr. *76*, ex BPI; Parc du Lébisey, près Caen, Herb. Barbey-Boissier 2425, ex BPI (as *Labrella periclymeni*) (Fig. 1).

On *L. utahensis* S. Wats. CANADA: **British Columbia**: Salmo, DAOM 6777;* Copper Mtn. 6800 ft, near Nelson, J. A. Calder and D. B. O. Savile 11040, DAOM 44610; E. side of Menashee Pass 3900 ft, Calder and Savile 10112, DAOM 44612; 3 miles E. of Revelstoke, Calder and Savile 8778, DAOM 44611.* UNITED STATES: **Washington**: 8 miles NW. of Locke, Pend Oreille Co., BPI 55911, ex UC. **Idaho**: N. of Moscow Mts., DAOM 44613, ex BPI (host as *L. ciliata*). **Montana**: Many Glacier, Glacier National Park, comm. Lee Bonar, DAOM 44614; Bozeman, DAOM 44615, ex BPI (host as *L.* sp.). **Wyoming**: Museum 6700 ft, Grand Teton National Park; Solh., Mycofl. Saximont. Exs. *292*, ex UC (Fig. 2).

In 1875, Berkeley and Broome described *Leptothyrium pictum* on *Lonicera* sp. from Glamis (Scotland). Although the host was not specifically determined, it is evident that this fungus is a synonym of *Lept. periclymeni* for Grove (5) lists the type locality along with several others in the British Isles under *Lon. periclymenum*. *Gloeosporium lonicerae* J. W. Ellis was also described on the same host.

Bäumler, who determined the specimen issued under Krypt. Exs. ed. Mus. Palat. Vindebon *835*, includes *Phyllosticta vossii* Thuem. (14, 15) as a synonym of *Lept. periclymeni*. The description suggests a fungus very similar to the latter in gross appearance of the pycnidia, but the description and figures of the spores do not fit those of *Lept. periclymeni*. I am indebted to Dr. G. R. Bisby, Commonwealth Mycological Institute, for a transcription of the description and a tracing of the figures.

Although the average dimensions of the spores of *Lept. periclymeni* on *Lonicera utahensis* are very similar to those of the typical variety, they have

*Canadian specimens so marked are referable to forma *ebractulata* (Rydb.) St. John.

in general an allantoid, rather than a falcate, outline when seen in side view. Future study may warrant placing this fungus in a distinct variety.

According to Saccardo (13) *Lept. periclymeni* var. *hispanica* Gz. Frag. was erected to accommodate a small-spored variety, collected on *Lon. hispanica* in Spain. The spores given as 12–20×6–8 μ are slightly shorter than the typical variety. No specimen has been seen.

LEPTOTHYRIUM PERICLYMENI var. **xylostei** (Pass.) comb. n.

Depazea xylostei Pass. in Rabh., F. Europ. *2220* (1877).

Labrella xylostei Fautr., Rev. Myc. **17**, 168 (1893); Allescher in Rabh., Krypt.-Fl. **1** (7), 364 (1901).

Colletotrichella xylostei (Fautr.) Höhn., Ber. Deut. Botan. Ges., **37**, 106 (1919).

Spores continuous, stouter than in the typical variety, 17–34.5 (38.5) × 5.5–13 μ.

Type.—On *Lonicera xylosteum* L.; prope Parmam, Sept. 1875, G. Passerini, Rabh., F. Europ. *2220*.

Specimens Examined

On *Lonicera hispida* Pall. EUROPE: **Czechoslovakia**: Bohemia: bei Turnau [Turnov] (cult.), Kab. & Bub., F. imperf. exs. *28*, ex BPI.

On *Lonicera xylosteum* L. EUROPE: **Denmark**: Moensklint, ex DAO. **Sweden**: Upland: Håga, prope Upsala, Vestergren, Microm. rar. sel. *736*, ex BPI; Harjedalen, Linsälls soeken, Glöte pa Nolåsen, DAOM 44625 ex DAO. **Germany**: Thüringia: Kernberge bei Jena, Sydow, Mycoth. germ. *2213*, ex BPI; Eisenach am Mädelstein, Migula, Cryptogam. Germ., Austr. et Helv. exs. *284*, ex BPI; Bavaria: Steinberg, Hersbruck, Herb. K. Starcs, Fl. bavar. 1287, DAOM 20229. **Czechoslovakia**: Mähr.-Weisskirchen: "Svrcov", Petrak, Fl. bohem. et morav. exs., II. Serie, 1. Abt., Lfg. 25, Nr. *1233*, ex BPI; Moravia: Tisnov [Tischnowitz] ex BPI. **Italy**: Parma, Rabh. F. Europ. *2220*, ex BPI (as *Depazea xylostei*); Susigana (Treviso), Sacc., Mycoth. veneta *964*, ex BPI (as *L. pictum*); Vallombrosa (Firenze), D. Sacc., Mycoth. ital. *565*. **Hungary**: bei Pressburg, Bratislava, Czechoslovakia, Linh. F. hung. *474* (Fig. 3). **Russia**: Berdicino, prov. Jaroslawl, Tranz. & Serebrianikow, Mycoth. ross. *292*, ex BPI; Toula [Tula], ex Herb. Inst. Myc. & Phytopath. Jaczewski, Petropolis, ex BPI.

The type of *Labrella xylostei* Fautr. was issued according to von Höhnel (6) in Roum., F. sel. exs. *6840*. The specimen has not been seen but Fautrey's figures, copied by Allescher (1), certainly suggests that *Labrella xylostei* is a synonym of *Lept. periclymeni* var. *xylostei*.

The collection of *Leptothyrium periclymeni* var. *xylostei* on cultivated plants of *Lon. hispida* at a forestry station near Turnov, Czechoslovakia, is of interest for the natural range of the host according to Rehder (11) is from Turkestan to West China.

KABATIA LONICERAE (Harkn.) Höhn., Ber. Deut. Botan. Ges. **37**, 106 (1919), var. LONICERAE.

Marsonia lonicerae Harkn., Calif. Acad. Sci. Bull. **1**, 31 (1884).

Gloeosporium lonicerae Harkn. ex Ell. & Ev., Journ. Myc. **1**, 116 (1885).

Pycnidia black; spores one-septate, 27–31.5 (34.5) × 5.5–8.5 μ, apical cell 7–14.5, basal cell 13–20.5 μ.

Type.—On *Lonicerae conjugialis* Kellogg, summit of Sierra Nevada, Calif., Aug. 1883, Harkness.

Specimen Examined

On *Lonicera conjugialis* Kellogg. UNITED STATES: **California**: summit of Sierra Nevada, ex Ellis Collection in NY (host as *L. conjugalis*) (Fig. 4).

Unlike most of the fungi recorded here, *Kabatia lonicerae* var. *lonicerae* is only known from a single collection made in California although Rehder (11) gives the range of the host as Washington to California and Nevada.

KABATIA LONICERAE var. **americana** (Ell. & Ev.) comb. n.

Leptothyrium periclymeni Desm. var. *americanum* Ell. & Ev., J. Mycology, **8**, 116 (1890).

Spores retort-shaped, usually shorter than the typical variety, 14–30×5–10 μ, apical cell frequently awl-shaped, (4) 5.5–12 (15.5), basal cell (11.5) 14.5–19.5 μ.

Type.—On *Lonicera* sp. (*L. canadensis* Marsh.), Parry Sound, Ontario, 1 July 1890, J. Dearness.

Specimens Examined

On *Lonicera canadensis* Marsh. CANADA: **Nova Scotia**: Truro, DAOM 1927; Kentville, DAOM 26147; New Minas, Kings Co., ex KP 1856. **Quebec**: St. Alphonse de Caplan, DAOM 12162; Kelly's Camp, Grand Cascapedia, DAOM 4607, 7330; L. Disparu, Ste. Anne de la Pocatière, DAOM 20837; St. Felicien, DAOM 6893; Bowker L., Sherbrooke Co., DAOM 1842; Stansted, DAOM 44617; Ile Perrot, DAOM 18613; W. of Danford L., DAOM 19590; Hermit Trail, near Pink L., DAOM 18612; Old Chelsea, DAOM 44618 ex DAO. **Ontario**: Brant Co.: Oakland Swamp, ex TRTC 20428; Haliburton Co.: Gull L., ex TRTC 23346, Pancake Camp, Batchawana Bay, ex TRTC 9236; L. Temagami: Bear I., Cain 1217, ex TRTC, Matagama Point, Cain 688 ex TRTC, Loon L. Portage, ex TRTC 1125 (as *Marssonia lonicerae*); Parry Sound, DAOM 44621, ex Herb. Dearness (co-type) (Fig. 5). UNITED STATES: **New York**: Stewarts Camp, DAOM 2298; Ithaca, N.Y., Barth. F. Col. *2527* (as *Lept. periclymeni*) (Fig. 6). **Michigan**: near Harbour Springs, DAOM 20526. **Wisconsin**: Ellison Bay, F. Wis. Exs. *13*, ex UC (as *L. periclymeni*).

Some authors would reject *Lept. periclymeni* var. *americanum* as a *nomen confusum*, because Ellis and Everhart intended to include under the variety both *Marsonia lonicerae* Harkn. and a fungus to hand from Canada. This new variety differed from the European *Lept. periclymeni* by the spores being septate. However, the authors noted that the spores of the fungus

on *Lon.* (*canadensis*) were smaller than those of the California fungus. Moreover, they indicate the two specimens upon which they based their concept. I have chosen to revive it for the fungus on *Lon. canadensis*, which was named by them for the first time.

Kabatia lonicerae var. *americana* appears to be one of the most widely distributed of these fungi as it occurs on *Lon. canadensis* from Nova Scotia west to Wisconsin and south to Pennsylvania, over the range of the host except along its western limits.

KABATIA LONICERAE var. **latemarensis** (Bubák) comb. n.

Kabatia latemarensis Bubák, Oesterr. Botan. Z. **54**, 3 (1904).

Spores longer than in the typical variety, 25.5–43×5.5–7 μ, apical cell relatively long, 7–21 μ, basal cell 16–22 μ.

Type.—On "*Lonicera xylosteum* L. Süd-Tirol auf Wiesen und in Wäldern unter dem Latemargebirge am Costalungapass (ca. 1650 m) in den Dolomitalpen. 10 Juli 1903, leg Kabát."

Specimens Examined

On *Lonicera coerulea* L. EUROPE: **Italy**: "Unter dem Latemargebirge am Costalungapass (ca. 1600 m) in den Dolomitalpen, Südtirol," Kab. & Bub., F. imperf. exs. *180*, ex BPI (Fig. 7). **Germany**: Oberammergau: Laineufer, All. & Schnabl. F. bavar. *280*, ex BPI (as *Lept. periclymeni*).

Undoubtedly F. imperf. exs. *180* was intended as the type collection and the host is *Lon. coerulea* and not *Lon. xylosteum* as given in the original description. According to Müller (9) the authors noted the change when they described *K. mirabilis*.

KABATIA LONICERAE var. **involucratae** var. n.

Sporae magnae, uniseptatae, 33–53 (57)×5.5×11.5 μ; loculo superiore 7.5–24 μ, loculo inferiore 24–42.5 μ.

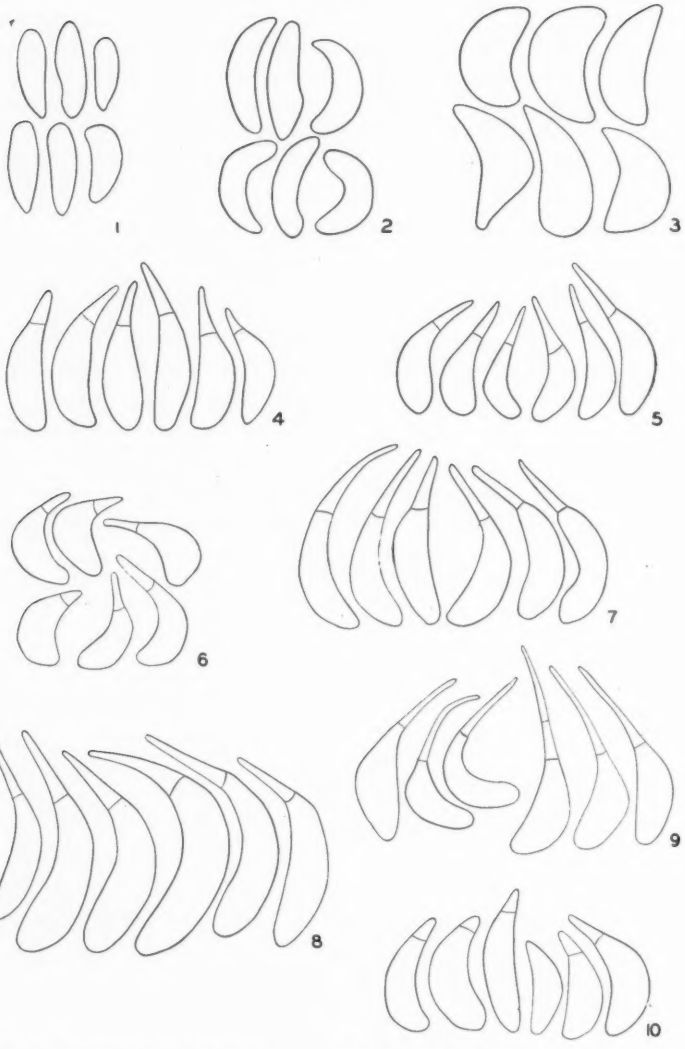
Type.—On *Lonicera involucrata* (Richards.) Banks; Coulter, Colo., Aug. 1904, E. Bethel, Ell. & Ev., F. Col. *2123* (issued as *Marsonia lonicerae* Hark.).

Specimens Examined

CANADA: **British Columbia**: Peachland, DAOM 3338; Hedley, DAOM 3337; Fort St. James, DAOM 5004 (Fig. 8); 2.5 miles S. of Kimberley 3200 ft, Calder and Savile 11372; Copper Mtn. 6800 ft near Nelson, Calder and Savile 11018; Yoho River Valley, Yoho National Park, Calder and Savile 12168. **Alberta**: Lake Louise, DAOM 44626; Waterton Lakes, DAOM 2236.

UNITED STATES: **Colorado**: Coulter, Ell. & Ev., F. Col. *2123* in DAOM (type). **Wyoming**: Medicine Bow Lodge, Medicine Bow Mountains, Carbon Co., Solh., Mycofl. Saximont. Exs. *387*, ex UC (as *K. mirabilis*).

Of the three varieties distinguished from typical *Kabatia lonicerae*, var. *involucratae* is very easily separated on account of the distinctly large size of its spores. The other two varieties are very close to the typical variety, but in var. *americana* the spores are usually short and broad with an awl-like



FIGS. 1–10. Spores of *Leptothyrium* and *Kabatia*. Figs. 1–2. *Lept. periclymeni* var. *periclymeni* from *Lon. periclymenum* and *Lon. utahensis*. Fig. 3. *Lept. periclymeni* var. *xylostei* from *Lon. xylosteum*. Fig. 4. *K. lonicerae* var. *lonicerae* (type). Figs. 5–6. *K. lonicerae* var. *americana* from Parry Sound, Ont. (co-type) and Ithaca, N.Y. Fig. 7. *K. lonicerae* var. *latemarensis* (type). Fig. 8. *K. lonicerae* var. *involucratae*. Fig. 9. *K. mirabilis* var. *mirabilis* from *Lon. nigra* (authentic specimen). Fig. 10. *K. mirabilis* var. *oblongifoliae* (type). ×820.

apical cell set at varying angles to the large basal cell. In var. *latemarensis* the septum of the spore is more nearly equatorial, resulting in the apical cell being relatively longer than in the other varieties.

KABATIA MIRABILIS Bubak, Oesterr. Botan. Z. **55**, 241 (1905), var. MIRABILIS.

Pycnidia yellowish buff, spores 21.5–45.5×6.5–13 μ, apical cell 11.5–21.5 μ, basal cell 17–27 μ.

Type.—On *Lonicera nigra* L., "im Steingerolle über grossen Karrersee unter Latemargebirge (ca. 1550 m) im Eggental, am 25. July 1904, leg. E. Kabát."

Specimens Examined

On *Lonicera alpigena* L. EUROPE: **Italy**: Süd-Tirol: "am Wege von Plan zum Grödnerjoch, Gröden," Kab. & Bub., F. imperf. exs. *726*, ex BPI.

On *Lonicera nigra* L. EUROPE: **Italy**: Südtirol-Setten: "uber dem Karrersee im Latemargebirge der Dolomitalpen," Kab. & Bub., F. imperf. exs. *475*, ex BPI (Fig. 9); Südtirol: "Bei Wolkenstein in Grödner Tal ca. 1650 m." Jaap, F. sel. exs. *290*, ex BPI. **Switzerland**: Graubünden, Fürstenalp, 1800 m, Vestergren, Microm. rar. sel. *1435*, ex BPI.

On *Lonicera xylosteum* L. [?] EUROPE: **Czechoslovakia**: Hochgesenke, Ramsau, Petrak, Fl. Bohem. et Morav. exs. Lfg. 39, Nr. *1927*, ex BPI (as *K. latemarensis*).

KABATIA MIRABILIS var. **oblongifoliae** var. n.

Pycnidia pallidissime lutea, inconspicua, dispersa in maculis pallide brunneis; sporae hyalinae uniseptatae, 19–33×7–10 μ; loculo superiore 3–6 μ, loculo inferiore 16–27 μ.

Type.—On *Lonicera oblongifolia* (Goldie) Hook. var. *oblongifolia*, 3 miles SE. of Casselman, Ont., 4 July 1952. D. B. O. Savile 3135 and I. L. Conners, DAOM 44627.

Specimens Examined

On *Lonicera oblongifolia* (Goldie) Hook. CANADA: **Ontario**: Casselman, near Ottawa, June 4, 1892, TRT 1611 DAOM 60440; 3 miles SE. of Casselman, Cody 6399, Calder and Savile, DAOM 28559 and Savile 3135 and Conners, DAOM 44627 (type) (Fig. 10); Carleton Co.: Mud Pond, March Twp., Savile 3188 and Parmelee, DAOM 44628; Brant Co.: Hatchley, July 25, 1884, TRT 1610 DAOM 60441; Bruce Co.: Allenford, TRT 81973 DAOM 60444, French Bay, TRT 75069 DAOM 60442, Gould L., TRT 75068 DAOM 60443, Howdenvale, TRT 34888 and Wingham, June 20, 1890, TRT 1612 DAOM 60439; Thunder Bay Distr.: Peninsula, TRT 56784.* UNITED STATES: **New York**: Bergen Swamp, CUP 10055 (as *Lept. periclymeni*). **Wisconsin**: Wind L., Racine Co. June 14, 1888, J. J. Davis ex WIS and June 29, 1903, J. J. Davis ex WIS.

It is of interest to note that the eight records from the University of Toronto Phanerogamic Herbarium were secured from leaves showing leaf spots removed by Dr. R. F. Cain from 12 separate collections of *L. oblongifolia*

*All *L. oblongifolia* var. *oblongifolia*.

Collections of *L. oblongifolia* in DAO failed to provide any additional records, but single records of two other entities were obtained from phanerogamic specimens of other species of *Lonicera*.

It has been suggested by von Höhnel (7) that *Marssonia staritzii* Bres. (12) described on *Lonicera tatarica* L. might be a member of this group. The type has not been seen. A specimen identified as this species on *L. tatarica* collected 27 June 1890 by G. Delacroix at Maines (Deux-Sèvres) ex BPI "comm. E. Griffon 12/8/08" showed Leptothyrium-like pycnidia, but no spores were found.

Dr. Bisby kindly loaned a leaf from a specimen labelled "*Pseudogloeosporium periclymeni* (Sacc.) Jacz." on *Lonicera tatarica* L. collected in Siberia, Distr. Tara, by Zillig 28 viii 25, ex Phytopath. Lab., Siberian Agric. Academy USSR Omsk, IMI 16248. The specimen was in very poor fruit, but the fungus is *Leptothyrium periclymeni* var. *xylostei*. Comparison of the leaf with those on specimens of *L. tatarica* in the phanerogamic herbarium, Ottawa, makes it appear extremely doubtful that the host was correctly determined. Until more adequate material is available, *Lonicera tatarica* must be considered a very doubtful host of *Lept. periclymeni* or a *Kabatia*.

Discussion

The three fungi discussed in this paper appear to occur on a relatively few species in the genus *Lonicera*. According to Rehder (11), the host genus is comprised of about 100 species, whereas these fungi have been recorded on only 12 species. The relation of the parasitized species within the genus is conveniently shown by arranging them by subgenus and section and indicating their respective parasite opposite. The number in parentheses is the number of species in each division.

Subgenus CHAMAECERASUS (82)	
Section ISOXYLOSTEUM (8)	
Section ISIKA (53)	
canadensis	*K. lonicerae* var. *americana*
utahensis	*L. periclymeni* var. *periclymeni*
coerulea	*K. lonicerae* var. *latemarensis*
hispida	*L. periclymeni* var. *xylostei*
involucrata	*K. lonicerae* var. *involucratae*
oblongifolia	*K. mirabilis* var. *oblongifoliae*
alpigena	*K. mirabilis* var. *mirabilis*
conjugialis	*K. lonicerae* var. *lonicerae*
nigra	*K. mirabilis* var. *mirabilis*
Section COELOXYLOSTEUM (13)	
xylosteum	*L. periclymeni* var. *xylostei* and ? *K. mirabilis* var. *mirabilis*
Section NINTOOA (8)	
Subgenus PERICLYMENUM (20)	
caprifolium	*L. periclymeni* var. *periclymeni*
periclymenum	*L. periclymeni* var. *periclymeni*

It seems quite possible that these fungi are highly specialized to their hosts and this limitation has resulted in their evolving into morphologically distinguishable varieties. As here treated, *Leptothyrium periclymeni* attacks species in both subgenera of *Lonicera*. On the other hand, the two species of

Kabatia are almost, if not entirely, confined to the section Isika of the subgenus Chamaecerasus although they occur on more individual species than the first fungus. This observation suggests that *Leptothyrium periclymeni* is the oldest species of the group and that the *Kabatia* species are the younger members that may still be undergoing change, possibly on account of greater speciation in the section Isika than elsewhere in the genus *Lonicera*.

These fungi apparently are confined to Europe and North America. Lee Ling (8) fails to record them from western China and they are unknown to Dr. Naohide Hiratsuka (in litt.) in Japan. The occurrence of *Lept. periclymeni* var. *xylostei* on a host that was labelled *Lon. tatarica* but doubtfully this species from Tara district in Western Siberia provides the only record from Asia.

The restricted range of the pathogens in comparison with that of the hosts may merely be due to lack of collecting. On the other hand, it may be the various host species comprise several distinct varieties that are not all natural hosts of these pathogenic fungi. The widely occurring Eurasian species, *Lon. coerulea*, *nigra*, and *xylosteum*, would otherwise provide suitable hosts for these fungi throughout Asia, including Japan. In North America, *Kabatia lonicerae* var. *involucratae* is known from British Columbia, Alberta, and the western United States, whereas the host *Lon. involucrata* is well represented in the DAO herbarium, not only from the western provinces but also from Manitoba to Quebec.

Acknowledgments

This paper was made possible as a result of assistance given by the late Dr. G. R. Bisby in providing critical information and by Mr. John A. Stevenson, Beltsville, Md., and the curators of the other herbaria cited through the loan of specimens. I am also indebted to Dr. D. B. O. Savile for making fresh collections of *Kabatia mirabilis* on *Lonicera oblongifolia*.

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TRANSLOCATION OF C^{14}-LABELLED AMINO ACIDS AND AMIDES IN THE STEMS OF YOUNG SOYBEAN PLANTS[1]

C. D. Nelson and Paul R. Gorham

Abstract

Translocation of each of seven amino acids and three amides was measured for periods of 5 minutes or less after introduction through the cut petiole of a primary leaf. The compounds used were asparagine, urea, glutamic acid, glutamine, glycine, norleucine, arginine, serine, alanine, and aspartic acid. During the short times of these experiments it was found that each compound was translocated downward as such. The amount of carbon-14 in the stem decreased logarithmically from the point of introduction. Each compound was translocated with unchanged velocity past a short section of stem killed with steam. There was no translocation of aspartic acid through a stem that had an entire internode killed with steam. Potassium cyanide (10^{-2} *M*) did not inhibit the velocity of translocation of any of the compounds although the logarithmic pattern of distribution of arginine was altered. The minimum velocity of translocation was different for each compound and varied between 350 cm per hour for asparagine and 1400 cm per hour for aspartic acid.

Introduction

The technique of introducing C^{14}-labelled compounds into the cut petiole of a primary leaf has been used to study translocation of sugars (9) and amino acids (10). Using this technique it has been possible to study translocation of physiological amounts of a single compound, in short enough periods of time to eliminate metabolic conversion to other compounds.

In a previous paper (10) the effect of the aging of leaves and the influence of the roots on the pattern of distribution of 10 amino acids or amides was described. The present paper describes the effect of steam girdling and potassium cyanide on the downward translocation of the same 10 compounds. In addition, the minimum velocity of translocation of all 10 compounds is reported. Part of this work has been previously reported (11).

Materials and Methods

Plants of *Glycine max* L. variety Comet were grown in vermiculite without nutrient as previously described (10). Plants with one fully expanded trifoliate leaf, 20 to 24 days old (stage I, cf. 8), were used except in the experiments determining velocity where the plants had fully expanded primary leaves but no trifoliate leaves.

The following 10 amino acids or amides were used: L-asparagine-G-C^{14}, urea-C^{14}, DL-glutamic acid-2-C^{14}, L-glutamine-G-C^{14}, DL-glycine-1-C^{14}, L-arginine-G-C^{14}, DL-serine-3-C^{14}, DL-alanine-1-C^{14}, DL-aspartic acid-4-C^{14}, and DL-norleucine-1-C^{14}. The specific activity of each compound and the concentration of solution administered to the plants were the same as described in Table I of the previous paper (10).

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The plants were transferred from the growth chamber to the laboratory (illumination, 300 ft-c) for translocation experiments. As before, all solutions were administered for 1 to 5 minutes through the cut petiole stump of one primary leaf. The stem between the primary node and the root was cut into sections 1 to 4 cm long (cf. Fig. 1). Each piece was assayed for C^{14} content and treated to two-dimensional paper chromatography as previously described (8).

Girdling was accomplished by steaming (9). Except where otherwise indicated, plants treated in this way were left in the laboratory overnight before use in experiments. After the period of "drying", the steamed section was brown and contracted to about half its original diameter.

In some experiments the aqueous solution of amino acid or amide contained 10^{-2} *M* potassium cyanide so that both compound and inhibitor could be administered simultaneously. In other experiments 10^{-2} *M* KCN was administered through the cut petiole for 30 minutes prior to administration of the test compound. No difference was observed between these two cyanide treatments.

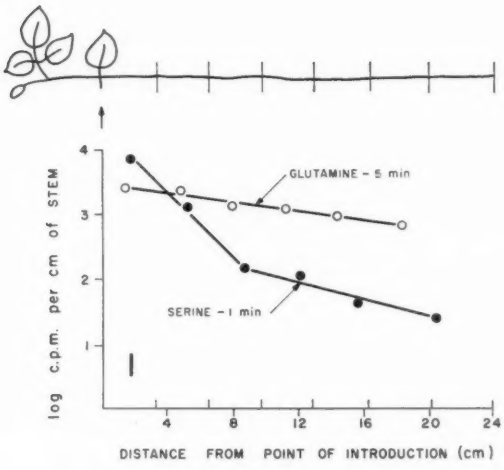
Each treatment with each of the 10 amino acids or amides was replicated at least three times.

Results

In translocation times of 5 minutes or less, chromatography and radioautography showed that the radioactivity in each stem section was present only in the compound administered. Therefore, in all the figures presented in this paper the measure of the radioactivity, expressed as c.p.m. per cm, is also a measure of the amount of the particular amino acid or amide translocated.

Translocation of Amino Acids in the Stems of Untreated Plants

If the logarithm of c.p.m. per cm of stem was plotted against distance in cm from the point of introduction two patterns of distribution of radioactivity were obtained as shown in Fig. 1. For example, the amount of labelled glutamine decreased logarithmically from the point of introduction after downward translocation for 5 minutes. Asparagine (Fig. 2), urea, glutamic acid, glycine, and arginine (Fig. 3) also had the same pattern of distribution in the stem as glutamine. Serine (Fig. 1), alanine, and aspartic acid had a different pattern of distribution. The amounts of these amino acids decreased logarithmically with a change in slope about half way down the stem. This "broken logarithmic curve" suggests that translocation in stems is very complex. Translocation in the conductive elements may be obscured by accumulation of amino acid by the surrounding tissues. If the rate of accumulation of tracer was slower than the rate of translocation then a broken curve would result. This pattern may be due also to a rapid translocation of amino acid in one tissue and a slow translocation in a second tissue. The patterns obtained for distribution of labelled amino acid or amide in the stem may have little to do with translocation as such.



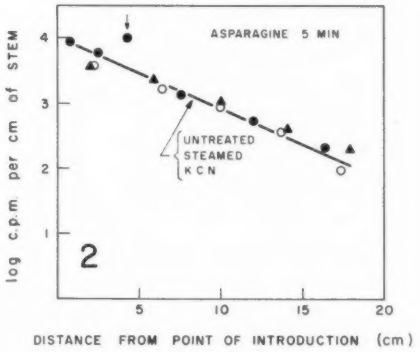
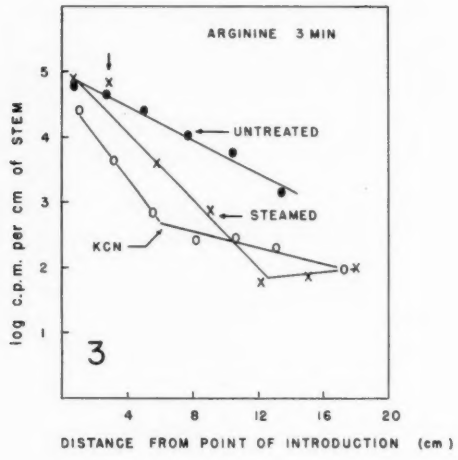


FIG. 1. Downward translocation of glutamine and serine in soybean stems. Arrow indicates point of introduction of the labelled compound.

FIG. 2. Downward translocation of asparagine in soybean stems. ▲, untreated; ○, 30 min pretreatment with KCN; ●, steamed. Arrow indicates the steamed section of stem where asparagine accumulated.

Effect of Cyanide and Steam Girdling on Translocation

In Fig. 2 data are plotted for the downward translocation of asparagine after a period of 5 minutes for a normal untreated plant, for a plant that was treated with 10^{-2} *M* KCN, and for a plant that had a section killed with steam. Neither of these two treatments had any effect on the velocity of translocation of this compound. It should be noted that at the same time that asparagine passed through, it also accumulated in the steamed section of stem. Neither treatment affects any other compound except arginine.



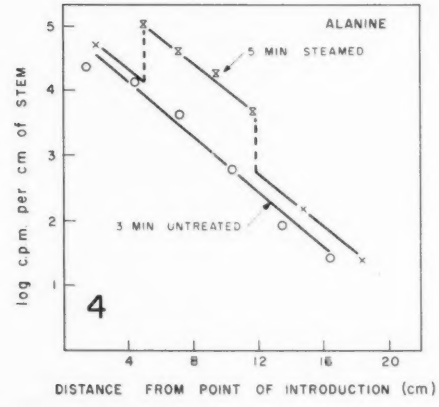


FIG. 3. Downward translocation of arginine in soybean stems. Arrow indicates the steamed section of stem where arginine accumulated.
FIG. 4. Downward translocation of alanine in the 1st internode of soybean stems. ⧖ indicates steamed sections of stem.

The data for arginine are plotted in Fig. 3. In the untreated plant the amount of arginine, like that of all other amino acids, decreased logarithmically from the point of introduction. Both treatment with KCN and killing a stem section with steam changed the distribution of arginine in the stem so that the pattern closely resembled that for serine (Fig. 1). There is an indication that KCN was more effective in this regard. Again two interpretations are possible. Cyanide and steaming may have inhibited accumulation by the tissues around the conductive elements without inhibiting translocation. Alternatively, if there was translocation in two different

tissues, translocation in one tissue was inhibited while that in the second tissue was not.

Although the translocated amino acid passed through a section of stem 1 to 2 cm long it was of interest to see how much of the stem could be killed without affecting translocation. In Fig. 4 data are plotted for alanine administered to an untreated plant and to a plant that had 7 cm of the internode between the primary and cotyledonary nodes killed with steam. The steamed plant was allowed to "dry out" before the experiment. Since the tops of plants treated in this way remained turgid for several days, movement of water from roots to leaves was apparently unaffected. It is evident that the amount of alanine decreased logarithmically from the point of introduction with an accumulation in the steamed portion of the internode. Since the same slopes were observed in both the steamed section and the unsteamed section of the steam-girdled stem and also in the untreated stem, alanine was translocated through the steamed stem without any change in velocity. For this reason, steaming probably did not affect the pathway of translocation. In other words, alanine was being translocated in non-living cells of the normal, unsteamed stem, possibly the xylem vessels.

Table I shows results for translocation of aspartic acid when the whole internode between the primary and cotyledonary nodes was killed with steam. These plants were treated in such a way that the primary node and about 0.5 cm of stem below the node were left undamaged. Aspartic acid was administered to some plants immediately after steaming (designated, steamed wet) and to others after standing overnight in the laboratory (steamed dry). The tops of plants treated in this way became flaccid within 2 hours indicating that movement of water from roots to shoots was seriously inhibited. In the steamed wet plants there was no translocation of aspartic acid past the first section of stem which contained the short undamaged section. In the steamed

TABLE I

Distribution of C^{14} in stems of soybean seedlings after downward translocation of aspartic acid-C^{14} for 3 minutes

Untreated		1st internode, steamed, wet		1st internode, steamed, dry	
Length, cm	Total activity per cm, c.p.m. $\times 10^3$	Length, cm	Total activity per cm, c.p.m. $\times 10^3$	Length, cm	Total activity per cm, c.p.m. $\times 10^3$
*	*	*	*	*	*
3.0	3.76	2.9	3.06	2.1	14.78
3.0	2.92	2.8	0	2.5	4.20
3.0	2.13	3.0	0	2.7	0.23
3.5	1.25	3.0	0	2.4	0
3.5	0.52	2.8	0	2.6	0
3.0	0.25	3.9	0	2.8	0
†	†	†	†	†	†
3.0	0.09	3.4	0	3.8	0
3.0	0.09	3.6	0	3.5	0

*Indicates position of primary node.
†Indicates position of cotyledon node.

dry plants there was movement of aspartic acid through 7 cm of stem. This is essentially what one would expect in a chromatogram of aqueous aspartic acid over dry cellulose.

From all of these experiments using plants with steamed stems it can be concluded that translocation took place through a killed section of stem if this section was not too long and if the movement of water from roots to shoots was not affected.

Velocity of Translocation of Amino Acids

Young plants with fully expanded primary leaves but without trifoliate leaves were used for determinations of velocities of translocation. The distance a compound moved in 1 minute was used to calculate the velocity. Since radioactivity must accumulate in any given stem section to a high enough level to permit detection the accuracy of the determination is dependent upon the sensitivity of the counter. Since a counter with an efficiency of 30% was used, radioactivity less than 15 absolute disintegrations per minute was not detected. There is a possibility that small amounts of amino acids were moved further down the stem than it was possible to detect. For this reason the calculations give only minimum velocities.

Table II shows the minimum velocities of downward translocation of the 10 amino acids and amides tested. Although the velocities varied from 368 cm per hour for asparagine to 1374 cm per hour for aspartic acid all of the velocities could be arranged in two groups: from 300 to 700 cm per hour and from 1000 to 1400 cm per hour. The three amino acids, serine, alanine and aspartic acid, that had the highest velocities of translocation also showed the "broken" logarithmic pattern of distribution described above. There was no correlation of molecular size or net valence charge with velocity. Glycine and urea, the smallest molecules with a net valence charge of 0, were translocated more slowly than norleucine, a larger molecule with the same charge. Aspartic and glutamic acids, both with a negative net valence charge, were translocated at quite different velocities, the smaller of the two molecules having the higher velocity.

TABLE II

Minimum velocity of downward translocation of amino acids and amides in soybean stems

Compound	Molecular weight	Net valence charge	Number of plants	Velocity,* cm per hour
Asparagine	132.1	0	4	368 ± 86
Urea	60.1	0	3	506 ± 111
Glutamic acid	147.1	−	4	563 ± 134
Glutamine	146.2	0	2	564 ± 24
Glycine	75.1	0	3	656 ± 144
Norleucine	131.2	0		1000 ± 8
Arginine	174.2	+	4	1157 ± 108
Serine	105.1	0	3	1317 ± 57
Alanine	89.1	0	3	1350 ± 68
Aspartic acid	133.1	−	3	1374 ± 51

*The figures after the ± signs are standard deviations.

It is possible that the accumulation of the test compound by the tissues surrounding the conductive elements determined the velocity of translocation. This may be studied with arginine since the accumulation of this compound in the stem is inhibited by KCN. Velocity measurements were made in the usual way using four stage 0 plants pretreated for 30 minutes with 10^{-2} *M* KCN. The velocity of translocation was 1100 ± 100 cm per hour, the same velocity that was found in untreated plants. It is concluded that the accumulation of a compound from the conductive elements had no effect on the velocity of translocation.

Discussion

Whenever the closed vascular system of a plant is cut changes in water relations are bound to occur. The xylem, which is under negative tension, may rapidly suck up solution until equilibrium is reached while the phloem, which is under positive tension, may exude liquid for long periods of time (3). After the petiole stump of soybean is first cut a small drop of exudate covers the cut end and is slowly reabsorbed into the plant. Exudation from the cut soybean petiole is only small in amount and does not continue for long periods as Crafts has described in cucurbits (3). The difficulties in studying translocation in an open system such as this are fully realized.

Two lines of evidence argue against this method of introduction being a typical xylem injection of liquid as Swanson suggests (13). First, translocation in this system is under physiological control. This is shown by the wide range of velocities obtained for the various compounds and by the fact that chilling the roots decreased the velocity of translocation of aspartic acid fivefold (10). Second, translocation of all compounds should be unaffected by steam girdling and KCN whereas in fact translocation of sucrose is inhibited by both treatments (9). If it is tentatively inferred that sucrose was rapidly accumulated into living cells near the site of entry into the plant system and subsequently translocated in living cells, then killing the living cells with steam or inhibiting their metabolic activity with KCN should allow xylem injection to take place.

Downward translocation of C^{14} in stems may be complicated by several factors. There is evidence to suggest that translocation may take place simultaneously in at least two different tissues. For example, Gage (5) has shown that photosynthate may move in the phloem at a velocity of about 100 cm per hour. Nelson *et al.* (12) have shown that photosynthate also moves in the xylem at a velocity of at least 3000 cm per hour. In addition, material may be moved out of the conductive elements and accumulated in the surrounding tissues (2, 6, 7, 13). The pattern of distribution of arginine in the cyanide-treated stem shows that it may be possible by selection of the proper inhibitor to study downward translocation without the complications of accumulation. The curves obtained by plotting total radioactivity per cm of stem against distance from point of introduction are the result of at least two and maybe more processes.

The experimental approach used in this study of amino acids has shown that under certain conditions organic molecules can be translocated with very high velocities. These velocities are in the same order of magnitude as those reported for "fast" photosynthate (12) and for the transmission of the "slow" excitation stimulus in *Mimosa* (1). Although the active substance has never been identified it is interesting to note that Fitting (4) tested about 1500 different chemical substances for activity in *Mimosa*. He found that only a few amino acids, alanine, glutamic acid, glycine, amino-*n*-butyric acid, and L-asparagine, were active. Of these alanine was found, in the present study, to have the highest velocity of translocation.

Acknowledgment

The technical assistance of J. G. Trepanier is gratefully acknowledged.

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PHYSIOLOGICAL CONTROL OF THE DISTRIBUTION OF TRANSLOCATED AMINO ACIDS AND AMIDES IN YOUNG SOYBEAN PLANTS[1]

C. D. Nelson and Paul R. Gorham

Abstract

Each of 10 C^{14}-labelled amino acids or amides was introduced into young soybean plants through the cut petiole of one primary leaf. The compounds used were asparagine, glutamine, urea, aspartic acid, glutamic acid, glycine, serine, alanine, norleucine, and arginine. The rates of uptake of all the solutions except arginine were in the range 1.0 to 1.5 μl per minute. After 1 to 5 minutes, the distribution of C^{14} throughout the plants was determined. Each amino acid was translocated as such without conversion to other compounds. From the point of introduction, translocation of each amino acid or amide was mainly downward toward the root; very little was translocated upward. The amount of asparagine or glutamine that was translocated into the primary leaf opposite the cut petiole increased as the leaf aged, while the amount of the other eight compounds decreased as the leaf aged. When asparagine and serine were administered together, serine moved into the young primary leaf while asparagine was excluded. Both excision of the roots and chilling the roots decreased the velocity of downward translocation of aspartic acid indicating that the roots exert a strong "demand" which favors translocation in a downward direction more than an upward direction in the stem.

Introduction

It often has been observed that the destination of translocated solutes is under physiological control. Solutes may be translocated to regions of high metabolic activity such as a stem tip (10), an elongating stem (15), or a developing root (6). Solutes may be accumulated in storage organs such as roots (3) or developing fruits (8). The normal physiological control over movement may be changed at infections which cause a mobilization of substrates from surrounding healthy tissue (16).

Previous studies (11) on the translocation of sugars administered through the cut petiole of a primary leaf of soybean showed that sucrose, glucose, and fructose were translocated mainly downward toward the root. In addition, cyanide and steam girdling inhibited the translocation of sucrose but not that of glucose or fructose. These studies have been extended to other compounds that originate in leaves during photosynthesis to see whether or not they are translocated in the same manner as the sugars. In the present paper the distribution of each of 10 C^{14}-labelled amino acids or amides together with the controlling influence of leaves and roots is described. A subsequent paper (12) describes the velocity of translocation of each of the 10 compounds and the effect of steam girdling and cyanide.

Materials and Methods

Plants of *Glycine max* L. var. Comet were grown in vermiculite under incandescent illumination at 22° C with a 16-hour day. The light intensity

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was 2000 ft-c measured at the height of the primary leaves. Plants were classified according to stage of development as follows:

Stage 0 plants, 17 to 20 days old with fully expanded primary leaves,

Stage I plants, with one fully expanded trifoliate leaf,

Stage II plants, with two fully expanded trifoliate leaves.

Since it took 4 days for full expansion of a trifoliate leaf the plants at intermediate stages of development were called 0i, 0ii, 0iii, Ii, Iii, Iiii.

Asparagine-G-C^{14} and glutamine-G-C^{14} were prepared using detached leaves of *Vicia faba* L. (13) and Swiss chard (1) respectively. The other radioactive compounds were obtained commercially as indicated in Table I. Since some of the compounds contained as many as 12 radioactive contaminants each was purified before use.

TABLE I

Source, specific activity, and concentration of solutions of the amino acids and amides used

Compound	Source*	Specific activity, mc per m*M*	Concentration of solution offered, m*M*
L-Asparagine-G-C^{14}	Bio. (14)	1.18	40
Urea-C^{14}	A.E.C.L.	1.66	72
DL-Glutamic acid-2-C^{14}	T.	0.52	77
L-Glutamine-G-C^{14}	Bio. (1)	0.50	32.4
DL-Glycine-1-C^{14}	A.E.C.L.	1.65	80
L-Arginine-G-C^{14}	A.E.C.L.	3.72	72.5
DL-Serine-3-C^{14}	A.E.C.L.	1.12	66.5
DL-Alanine-1-C^{14}	A.E.C.L.	3.54	76
DL-Aspartic acid-4-C^{14}	T.	0.75	67
DL-Norleucine-1-C^{14}	N.E.N.C.	1.13	59.5

*Bio.=biosynthesis;
A.E.C.L.=Atomic Energy of Canada Ltd., Commercial Products Division, Ottawa;
T.=Tracerlab, Waltham, Mass.;
N.E.N.C.=New England Nuclear Corp., Boston, Mass.

The plants were transferred to the laboratory (illumination, 300 ft-c) for translocation experiments. The blade of one primary leaf was excised in air with a razor. Then the petiole stump was pushed into a piece of capillary tubing containing an aqueous solution of labelled amino acid or amide (11). This was taken as zero time for each experiment. The concentrations of the solutions are shown in Table I. After 1 to 5 minutes the petiole was removed from the solution and the plant cut into pieces as indicated in Table III. Each piece was immediately ground in liquid nitrogen in a mortar and the frozen, ground tissue extracted with hot 80% aqueous ethanol. The ethanol-soluble extract was assayed for C^{14} and treated to two-dimensional paper chromatography and autoradiography as previously described (10).

All radioactivity determinations were made to within 3% standard error with a gas flow counter fitted with a "Micromil" window (Model D47, Nuclear, Chicago). The efficiency of the counter was 30%.

Experimental and Results

The soybean variety, Flambeau, was used in the previous experiments with sugars (10, 11). The improved variety, Comet, was used in the present experiments because it gave more uniform plants under our culture conditions. So that the translocation of the amino acids could be compared with that of the sugars, sucrose-C^{14} and glucose-C^{14} were administered to Comet plants with the same results as were found previously for Flambeau (11).

Form in which Amino Acids Were Translocated

Soybeans that had taken up C^{14}-labelled amino acids were divided into the parts that are indicated in Table III. Chromatography and autoradiography of the 80% ethanol-soluble extracts of each plant part showed that in periods of 5 minutes or less C^{14} was recovered only in the compound that was introduced. Also there was no radioactivity in the ethanol-insoluble residues. It is concluded that in these short times each amino acid tested was translocated as such.

Rate of Uptake of Solution Through the Cut Petiole of a Primary Leaf

Since the determination of C^{14} in each plant part was quantitative, the total amount of C^{14} recovered from any plant was also a measure of the total C^{14} introduced. To calculate the rate of uptake of solution it was assumed that the solute and the water were taken up together. From the specific activity of the *solution*, i.e. μc per μl, the total volume taken up was calculated and expressed as μl per minute.

The rates of uptake of aqueous serine solutions by several plants at different stages of development are shown in Table II. For all stages of development and for translocation times from 1 to 5 minutes the values varied between 1.15 and 1.54 μl per minute. Within these limits a doubling of the concentration of serine had no effect. However, when a translocation time of 10 minutes was used the rate of uptake was lower, indicating that after the first few minutes the rate probably decreased with time. For nine of the 10 amino acids or amides the values were within the range 1.0 to 1.5 μl per minute, the same range of values that was calculated for serine. Rates of uptake

TABLE II

Rate of uptake of aqueous serine solutions through the cut petiole of a primary leaf of young soybean plants

Stage of development	Concentration, μM	Time of translocation, min	Rate of uptake, μl per min
0	66.5	1	1.15
I	66.5	1	1.48
0ii	66.5	3	1.54
I	66.5	3	1.36
0i	133.0	5	1.38
0ii	66.5	5	1.39
Ii	66.5	5	1.25
0iii	66.5	10	0.81

TABLE III

Distribution of C^{14} in stage I soybean plants, after uptake of amino acids or amides through the cut petiole of a primary leaf

Plant part	Per cent of total C^{14} recovered									
	Asparagine, 5 min	Urea, 3 min	Glutamic acid, 3 min	Glutamine, 5 min	Glycine, 5 min	Arginine, 3 min	Serine, 3 min	Alanine, 1 min	Aspartic acid, 3 min	Norleucine, 3 min
Stem tip	0	0	0	0	0	0	0	0	0	0
Trifoliate leaf and node	trace	0	trace	0	0.1	0	0	0	0	trace
Stem between trifoliate and primary nodes	2.0	0.1	trace	0	1.6	1.0	13.1	1.5	0	10.0
Opposite primary leaf	17.3	0.7	trace	15.9	0.3	1.0	0.5	1.3	4.4	0.5
Treated petiole and node	50.0	52.8	31.0	35.4	41.0	30.0	21.9	75.5	18.2	27.0
Stem between primary and cotyledon nodes	30.2	46.3	63.0	47.4	56.1	68.0	64.6	21.5	72.5	72.0
Stem between cotyledon node and root	0.5	0.1	6.0	1.3	0.9	0	0	0.2	4.7	0.5
Root	0	0	0	0	0	0	0	0	0	0
Total C^{14} recovered (mμc)	242	175	121	87	759	683	604	432	161	201
Total C^{14}-amino acid recovered (μg)	27.1	6.3	35.6	25.5	34.5	32.0	56.7	10.9	29.0	25.3
Rate of uptake of solution (μl per min)	1.02	1.46	1.0	1.08	1.15	.85	1.35	1.6	1.08	1.0

of arginine, however, were consistently lower than those of the other nine amino acids or amides. It was observed previously (11) that sucrose, glucose, or fructose also was taken up at a rate of about 1.0 μl per minute. It is probable, therefore, that it is valid to assume that solute and water were taken up together. The assumption may not be valid for arginine, the only basic compound tested, or the rate of uptake of this amino acid may be lower.

Distribution of Translocated Amino Acids or Amides Throughout Soybean Plants

The distribution of each amino acid or amide was determined for at least four and sometimes as many as nine plants, at various stages of development from 0 to Iii. Although the distribution differed in detail with different compounds and with different plants the general pattern was the same. One typical example for each of the 10 amino acids or amides is shown in Table III.

In 3 to 5 minutes, between one-quarter and one-half of the labelled compound was recovered from the treated petiole and node. In all plants the bulk of each amino acid or amide was translocated downward and recovered in the stem between the primary and cotyledon nodes. Usually a small amount of labelled compound was recovered from the stem between the cotyledon node and the root. In short times no C^{14} could be detected in the root. However, in three experiments using labelled serine and a translocation time of 15 minutes considerable amounts of C^{14} were recovered from the root.

There was very little upward translocation of any of the introduced compounds. In short times of 5 minutes or less there was never any translocation to the stem tip. In some plants a small amount was recovered from the trifoliate leaf. Most of the amino acid or amide that was translocated upward was recovered from the stem between the trifoliate and cotyledonary nodes.

The amount of labelled compound recovered from the primary leaf opposite the cut petiole was different depending on the stage of development of the plant and the compound translocated (Table IV). There was no translocation of the amides, asparagine or glutamine, to the young primary leaf.

TABLE IV

Effect of stage of development (age) on translocation of asparagine, glutamine, and serine into the primary leaf of young soybean plants

Stage of development	C^{14} in primary leaf as per cent of C^{14} taken up by plant		
	Asparagine	Glutamine	Serine
0	—	0	25.2
0i	0	0.5	20.6
0ii	—	3.5	16.9
0iii	6.2	—	11.2
I	4.7	—	6.6
Ii	13.6	15.9	0.5
Iii	17.3	—	—

However, the amount of amide translocated to this leaf increased with age. Conversely, the amount of serine translocated to the young leaf was high and decreased with age. Results similar to those tabulated for serine were found for the other six amino acids and urea.

To check on the movement of serine into the primary leaf with a simultaneous exclusion of asparagine, a solution containing 33 m*M* serine and 20 m*M* asparagine was administered to the cut petiole of two stage 0 plants. After a translocation time of 5 minutes the primary leaves of the two plants contained 0.8 and 1.2 mμc of C^{14}; all of the radioactivity was recovered as serine.

The Effect of the Roots on the Translocation of Aspartic Acid

Since each amino acid was translocated mainly downward, it was possible that the roots exerted a "demand" which caused movement of compounds in this direction. To test this hypothesis the following experiments were carried out using aspartic acid. Stage Iii plants which translocated little or no aspartic acid to the primary leaf were chosen. In one experiment the roots were excised under water and the exposed stump of the stem kept in water during a translocation time of 3 minutes. In a second experiment the roots were chilled for 30 minutes by immersion in water at 5° C and kept in this condition during a translocation time of 3 minutes. Each treatment was carried out with three plants. The distribution of C^{14} in two plants, one for each treatment, and in an untreated plant is shown in Table V.

Both treatments reduced the rate of uptake of solution. Removing the roots increased the proportion of aspartic acid that was translocated upward. Chilling the roots, on the other hand, had little or no effect on the proportion that was translocated upward. However, in both treatments, aspartic acid was translocated to the stem tip. No translocation to the stem tip was

TABLE V

Distribution of C^{14} in stage Iii soybean plants, without roots and with chilled roots, after uptake of aspartic acid through the cut petiole of a primary leaf for the times indicated

Plant part	Per cent of total C^{14} recovered		
	Untreated, 1 min	Without roots, 3 min	Chilled roots, 3 min
Stem tip	0	1.8	0.4
Trifoliate leaf and node	0	0	0
Stem between trifoliate and primary nodes	7.2	20.6	3.7
Opposite primary leaf	0	0	3.8
Treated petiole and node	49.4	32.4	27.0
Stem between primary and cotyledon nodes	43.4	45.2	65.1
Stem between cotyledon node and root	0	0	0
Root	0	0	0
Total C^{14} recovered (mμc)	61	95	26
Total aspartic acid recovered (μg)	11.0	17.4	4.7
Rate of uptake of solution (μl per min)	1.23	0.64	0.18

found in untreated plants (cf. Table III). In addition, more aspartic acid was translocated to the primary leaf in the plant with chilled roots than in the plant without roots or in the untreated plants.

The distribution of aspartic acid was determined in the internode between the primary and cotyledon nodes. This was done by cutting the internode into pieces 1 to 3 cm long and assaying for C^{14} in the usual way. Results were calculated as the logarithm of mμc per cm of stem and plotted against distance (cm) from the point of introduction (Fig. 1). For purposes of comparing the treated plants that were allowed a translocation time of 3 minutes with an untreated plant with a translocation time of 1 minute the data were normalized. The amount of C^{14} per cm of stem in the first section of internode below the primary leaf was put equal to 100 and the amount of C^{14} in the other sections made proportional to this. Thus, in Fig. 1, the first section below the primary node contained 3.5 mμc in the untreated plant, 4.7 mμc in the plant without roots, and 2.0 mμc in the plant with chilled roots.

In the untreated plant and in the plant with chilled roots, the amount of C^{14} decreased logarithmically with the same slope from the point of introduction. The decrease was not logarithmic in the plant without roots. A velocity of translocation can be calculated from the distance aspartic acid moved in the given time. These velocities are shown in parentheses in Fig. 1. The velocity of translocation was 5 to 6 times faster in the untreated plant than in the treated plants. It is concluded that the roots did exert a "demand" which caused movement of aspartic acid downward in these experiments.

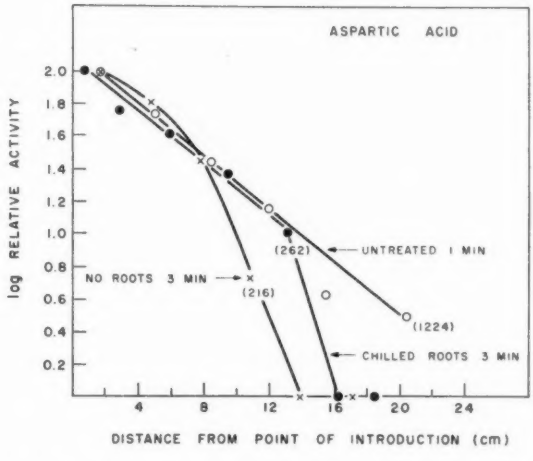

FIG. 1. Downward translocation of aspartic acid in the first internode of the stems of young soybean plants. Figures in parentheses are velocities of translocation in cm per hour.

Discussion

There are many instances of solutes being translocated predominantly downward from mature leaves. For example, carbon that is fixed in photosynthesis is translocated to the roots of soybean (10, 17), bean (15), and pumpkin (14). Chailakhyan (2) has shown that both vegetative and flowering shoots of red perilla are supplied by solutes moving from leaves above rather than below the shoots. The movement of C^{14}-labelled 2,4-D is mainly to the roots of cotton, bean (4), and wild morning glory (5) when this herbicide is applied as a spot on cotyledons or mature leaves. Nelson and Gorham have shown that the main movement of both sucrose and glucose is toward the roots when these sugars are applied through the cut petiole (11) or applied as a spot to the primary leaf (10). All 10 of the amino acids or amides tested in the present study moved mainly downward toward the root.

The experiments with chilled roots show that translocation takes place to regions of high metabolic activity (cf. Rabideau (15), Crafts (4)). This may be directly related to the absorbing power of roots as Pristupa and Kursanov believe (14) or it may be related to the capacity of roots for storage. However, it is interesting to note that when downward translocation of aspartic acid was decreased by chilling the roots there was translocation into the growing stem tip, another region of high metabolic activity. From the point of introduction, material was apparently "pulled" in three directions, to the roots, to the opposite primary leaf, and to the stem tip. Reducing the "pull" towards the roots by chilling or by removing the roots allowed material to be translocated to the stem tip. However, translocation was predominantly downward even in plants which had their roots removed.

The elongating stem of soybean is also a region of high metabolic activity acting to "pull" material downward. Rabideau and Burr (15) found that the amount of labelled photosynthate that accumulated in the stem of bean was correlated with the growth rate. Kursanov (7, 14) has postulated that there is a cycle of movement of material from leaves to roots and back to shoots. The present experiments show that material can be translocated to the shoots without first being moved to the roots.

It is difficult to explain why the amino acids were translocated to the young and not to the old primary leaf while the amides were translocated to the old and not to the young primary leaf. This indicates that although the conductive elements were present and functional in these young plants, the destination of the amino acid or amide was selectively controlled. If translocation is to a region of high metabolic activity then there must be a "demand" in the old primary leaf for the amides and not for the other amino acids. A similar "demand" for assimilates has been described for the growing shoots of red perilla by Chailakhyan and Butenko (2), who refer to "sucking activity of shoot tissue". In this connection it is interesting to note that in close to a hundred soybean plants analyzed in this study endogenous asparagine was never isolated by paper chromatography from primary leaves of all ages, while the concentration of serine was highest in young leaves.

If translocation of solutes is in accordance with the pressure flow mechanism (9) then all compounds should move more or less together. This mechanism cannot explain the translocation of amino acids and amides to the primary leaves observed in the present study.

Acknowledgment

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ANALYSIS OF THE HISTOCHEMICAL LOCALIZATION OF PEROXIDASE RELATED TO THE DIFFERENTIATION OF PLANT TISSUES[1]

D. S. VAN FLEET

Abstract

Peroxidase is detectible in all tissues but is most reactive in the basophilic cells of the histogens. Oxidation of applied phenols and aminophenols by peroxidase produces quinones and quinonediimines that are adsorbed by nucleic acids and other basophilic substances in the formative centers of primordia. Localized reactions for peroxidase occur in the axils of leaf primordia prior to bud formation and on the surface of apical meristems in a spiral pattern marking the points for the future development of leaf primordia. Peroxidase is detectible in advance of or accompanying cell division and declines after the division phase; decline of peroxidase at the end of the division phase is related to the increase of phenols, naphthols, and phenolases. Peroxidase declines in all tissues except the phloem; a continuous peroxidase system in the phloem connects primordia with adult tissue. The hypothesis is offered that the cellular units of the phloem peroxidase constitute a continuous system between primordia and adult tissue and is functional in catalyzing the reduction of hydrogen acceptors essential to cell division and the initiation of primordia.

Introduction

Enzyme differentiation precedes the anatomical signs for differentiation and it is axiomatic that enzyme systems characteristic of individual histogens and adult tissues develop either at the time of cell division or prior to this event. Hematin peroxidase is one of the first enzymes to be histochemically detectible in certain tissues (21). An analysis will now be presented of tests for peroxidase and of the distribution of peroxidase and the relation of peroxidase localization to cell division and differentiation.

Histochemical studies reported here were designed to reveal several postulates essential to an analysis of the significance of the localization of peroxidase. Preliminary peroxidase studies, as well as a consideration of earlier related research (21, 22, 24), suggested the following outline for the analysis. (*a*) Detection of the origin of peroxidase in relation to cell division and differentiation in primordia. (*b*) Relation of peroxidase increase or decline to the end of the division phase. (*c*) Relation of peroxidase decline to the development of other enzyme systems and to the increase of phenols, naphthols, and anthrols. (*d*) The significance of peroxidase reactions in basophilic centers.

The microspectroscopic ocular can be used to demonstrate that tissues showing the absorption bands for peroxidase also have the strongest peroxidase reactions with oxidizable indicators (21). With a monochromator as a light source and a photomultiplier photometer, spectrophotometric information was obtained on the distribution of peroxidase but it was necessary to use masses of tissue several millimeters in thickness (cf. Hartree (6)). The microspectrophotometer was used only in gross tissue studies of peroxidase,

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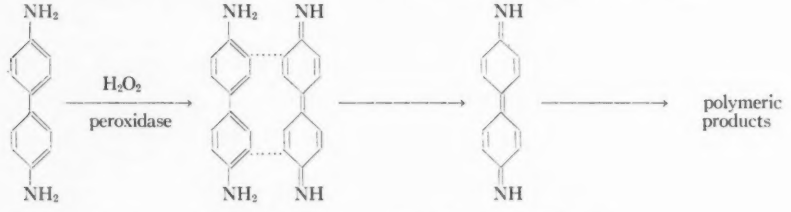
sufficient to show that the centers for peroxidase are the same as those indicated by the oxidation of benzidine and various phenols and aminophenols.

The most common indicator method for peroxidase detection employs benzidine and hydrogen peroxide as substrates. The oxidation of benzidine has been represented in histochemical methods (12, 9) as follows:



benzidine benzidine blue benzidine brown (diimine)

van Duijn (20) has evaluated benzidine oxidation by peroxidase and introduced improvements in the benzidine blue peroxidase method. van Duijn found that at a high salt concentration the blue intermediate product may be precipitated in the form of stable blue crystals; this was accomplished by the use of 5% ammonium chloride in the benzidine – hydrogen peroxide mixture.

The observations of van Duijn were confirmed and it was found that the site of benzidine blue precipitation is in part determined by salt concentration. However, without adding ammonium chloride, nickel ammonium sulphate, or other precipitating agents, it was found that benzidine blue is precipitated consistently in certain cells in early phases of a differentiation whereas in older cells oxidation and polymerization proceeded to benzidine brown. The work reported here is an analysis of the rate and extent of benzidine oxidation to benzidine blue or to benzidine brown in relation to cell division and differentiation as well as in the presence of precipitating agents.

Methods

Several indicators oxidizing by two steps to two kinds of end products were used in addition to benzidine. The common redox indicator 2,6-dichlorophenolindophenol (2,6-D) reduced to the colorless form with ascorbic acid was used in a modification of the method by Smith *et al.* (18). 2,6-D was applied to fresh sections with pH buffers to detect tissues oxidizing the 2,6-D to the blue form (first step) and those oxidizing to the brown form (second step) as in the secondary oxidation and polymerization of benzidine to the brown quinonediimine. In preliminary trials to determine general methods it was found that the peroxidase reaction in each tissue had a definite threshold level according to the tissue; for example, it was found that 8-aminoquinoline, not previously used as a peroxidase substrate, was oxidized to a non-diffusible end product only in those tissues having the most active peroxidase, whereas benzidine was oxidized to some extent in all tissues. Dimethylaniline and

p-anisidine were also used to separate activity of certain tissues (used previously for general peroxidase studies, cf. Saunders and Watson (15), Naylor and Saunders (11), Daniels and Saunders (4)). Many additional easily oxidizable phenols, those having hydroxyl groups in the ortho or para position, were employed to detect peroxidases and phenolases. Para-aminophenol, diphenylamine, para-methyoxyphenol, ortho-methyoxyphenol, para-quinone, catechol, and 2,4,6-trimethylaniline (mesidine) were used as peroxidase indicators.

The point at which phenolases appear in the histogens, in relation to the decline in peroxidase, was determined by applying these indicators to fresh sections without adding hydrogen peroxide to the preparation.

Ethylenedinitrilotetraacetic acid (EDTA), when applied to sections at the time of oxidation of benzidine to benzidine blue, stabilizes and precipitates the benzidine blue in crystalline form. When sections are pretreated with the chelating agent EDTA at higher concentrations there is a marked inhibition of peroxidase. Preliminary trials revealed that the effect of EDTA at concentrations greater than 150 p.p.m. inhibited peroxidase, but at the same time brought about an increase of phenolase activity (cf. Weinstein *et al.* (26)). Burström and Tullin (2) found that EDTA inhibits root growth at higher concentrations. The writer confirmed this fact in preliminary trials and applied EDTA at several concentration levels to induce a simultaneous inhibition of cell division and peroxidase. It was the premise of this work that EDTA may chelate and bind the active portion of peroxidase and disrupt one of the most significant systems in the regulation of the metabolism of cell division (cf. McDonald and Kaufman (10)). EDTA was supplied to the growing roots of *Allium sativum* at concentrations of 10^{-2} *M* to 10^{-5} *M* as the sodium salt.

The survey of tissues and the experiments were conducted on several unrelated plants in the following categories: the developing ovaries and embryo sacs of *Zea mays*; the wound meristems of *Coleus*, *Sansevieria*, *Cucurbita maxima*, and *Smilax hispida*; root tips of *Allium sativum*; roots from many varieties of bulbs; and the primary roots from several kinds of seeds.

Results

Benzidine, 2,6-Dichlorophenolindophenol, and 8-Aminoquinoline as Peroxidase Substrates

In the region of division in the meristem of the root the protoderm (proepidermis) gave the strongest reaction to peroxidase of any histogen (Fig. 1). The youngest cells of the protoderm oxidized benzidine to benzidine blue, the older cells at a distance of 2 mm back of the tip oxidized benzidine through the benzidine blue phase to benzidine brown, the quinonediimine. The benzidine blue end product was found in the locus of future cell divisions or in cells in active division but not in older resting cells. Oxidation and polymerization to benzidine brown was the rule in cells no longer in the meristem phase.

When previously oxidized benzidine blue was applied to meristems there was no localized absorption in the sites of cell division. Benzidine blue readily crystallized in dividing cells at the time of oxidation of benzidine to benzidine blue; but, if the benzidine was oxidized to benzidine blue by ferric salts and hydrogen peroxide and introduced to the tissue in its oxidized form as a mixture of basic benzidine blue and benzidine brown, there was no localized absorption by the basophilic cells. This was to be expected because of the insoluble nature of the oxidized benzidine, but the more soluble oxidation product of *p*-phenylenediamine also failed to reveal basophilic centers.

Excellent results were obtained with 2,6-D as an indicator for peroxidase. Leuco 2,6-D was oxidized to the blue form within 2 to 3 seconds in dividing cells of the proepidermis, proendodermis, and protophloem (Fig. 1 and Fig. 7), and the prometaxylem at the end of its division phase (Fig. 3). In older cells of these tissues a further oxidation to red-brown polymerized products was observed. Oxidation to the blue form obtained only in those cells in the region of protein synthesis and cell division (see also Fig. 2) and in the same centers in which benzidine was oxidized to benzidine blue.

Mesedine, and 8-aminoquinoline, oxidized more slowly than benzidine or 2,6-D. Whereas benzidine was oxidized by all tissues to some degree, 8-aminoquinoline was oxidized only by those tissues showing the most rapid and intense oxidation of benzidine. 8-Aminoquinoline was oxidized in young roots of *Allium* by the proepidermis (in less than 10 seconds) and more slowly by the protophloem (40 to 50 seconds) and oxidation in the proendodermis occurred only opposite the phloem poles.

Localization of Peroxidase in Primordia

Peroxidase was found to have its origin, prior to or accompanying cell division, in patterns related to the plane of cell division and the origin of centers of cell division. Proepidermis, proendodermis, protophloem, and pericycle of roots gave the most pronounced peroxidase reactions (Fig. 7). Using peroxidase tests these histogens were traced to the centers of their origin and peroxidase was found to be present in the histogen apex of the root. Cells in division giving rise to tissue systems had the most evident peroxidase localization. With increased distance from the root tip there was a decline in peroxidase in all tissues except the adult sieve tubes and companion cells.

Peroxidase was found to have a most distinctive localization in the developing metaxylem of roots. It was localized in cellular associations around the metaxylem with an appearance similar to the companion cells of the phloem (Fig. 3). These cells surrounding the metaxylem have been described by Resch (14) but with the use of peroxidase indicators a new insight into their content and appearance was obtained. Cytoplasmic units of peroxidase were found in accompanying companion cells in a series of units in columns that have yet to be determined. This phase in the life of the metaxylem system is short and occurs after cell division and prior to lignification.

Wound meristems were found to have a localization of peroxidase in advance of the establishment of the meristem. An increase of peroxidase was

observed in transition from rest to cell division in a pattern identical with the plane of ultimate cell division. Surrey (19) and Van Fleet (21, 24) found several enzyme systems characteristic of the early phases in the origin of a new meristem. One of the most readily detectible of these enzymes was peroxidase. When vascular bundles in *Smilax*, *Sansevieria*, and *Coleus* stems were severed by cutting a small sector from a young internode, a new meristem developed which gave rise to vascular tissue connecting the old severed bundles. Prior to the development of the new meristem the resting parenchymal cells, in the exact plane and pattern of the ultimate meristem, gave no reactions for peroxidase, but reactions for peroxidase appeared within a day or two following the initial stimulus to meristem formation, and several days in advance of the actual development of the meristem.

Oxidation of benzidine to benzidine blue was obtained in the central mass or corpus of the stem apex of *Asclepias* whereas oxidation and polymerization carried over to benzidine brown in the cortical or tunica portion. A general separation of outer and inner portions of the meristem was observed in several unrelated plants but the results were not of one kind. In some species a separation of cortex from stelar zones was observed but in others there was no difference in these two zones. In *Asclepias* the outer dermal layer of cells and one or two outer cortical layers gave a benzidine blue reaction and all other underlying cells gave a benzidine blue reaction.

The origin of buds in the stem apex of corn appeared to be prescribed in advance by development of peroxidase. Axillary loci for the origin of buds gave reactions for peroxidase prior to the actual cell divisions in bud formation (Figs. 4, 5, 6, 8, and 9). Centers for the ultimate development of buds appeared in a spiral pattern over the surface of the meristem (see Fig. 5), each center was made up of a small whorl of cells giving an immediate and heavier localization of benzidine blue than surrounding cells. Any damage to the meristems in the course of dissection gave irregular results and it must be emphasized that results obtained, as shown in Fig. 5, were subject to variables in method. Variation occurs in the receptivity of the tissue to the solvents, the substrate phenols, the threshold level of peroxidase activity, and the quantity of basophilic substances contributing to the adsorption of basic end products.

The eggs, and to a lesser extent the synergids, of the embryo sac in corn gave peroxidase reactions but the antipodal cells in the same embryo sacs failed to give a peroxidase reaction (Fig. 2). Nucellar cells immediately around the surface of the embryo sac were found to contain peroxidase particularly at the micropylar end of the embryo sac.

Peroxidase in the Phloem

Phloem mother cells have the strongest peroxidase reactions of the vascular tissue and give rise to sieve elements lacking nuclei and ostensibly "non-living" with weaker peroxidase reactions. The contents of the sieve tubes have been recognized for over 60 years as giving peroxidase reactions. Peroxidase content of the phloem is so characteristic as to be useful in detecting the tissue (Fig. 10). The non-living substance (cf. Currier, Esau, and Cheadle

(3)) of the mature sieve tube lacks nuclei but it contains some basophilic material possibly nucleic acid or phenolic acids readily holding the oxidized products of peroxidase reaction. Basophilic substances in the phloem adsorbing the quinones are not a part of the phloem callose.

It is important to any general view on peroxidase to emphasize the fact that both phloem and peroxidase develop before other systems in ontogeny. Peroxidase is one of the first enzyme systems detectible in a wound meristem prior to its division and Eschirch (5) has found phloem to be the first tissue, in some cases the only tissue, to develop in the regeneration of interrupted vascular tissue.

Peroxidase Loss and Cessation of Cell Division

In the first 1000 microns of the protoderm (proepidermis) benzidine was oxidized to benzidine blue; in the derived and older cells of the epidermis benzidine was oxidized to benzidine brown. Oxidation by peroxidase was rapid in young tissue (1–2 seconds) and slower (15 seconds) in cells at a distance of 2 mm from the tip of the meristem.

Ethylenedinitrilotetraacetic acid (EDTA) at high concentrations inhibited peroxidase reactions in all cells and it was found that roots stopped growing in EDTA in a concentration range of 10^{-2} *M* to 10^{-5} *M* (Figs. 13 and 14). It was clearly evident in the tests made that EDTA inhibited both peroxidase and cell division. In normal roots the peroxidase reaction was immediate and stopped at the intermediate benzidine blue oxidation step (Fig. 12). In roots treated with EDTA the benzidine blue oxidation products were not obtained and the reaction ran through to the polymerized benzidine brown product. In those roots in which growth had ceased, after 2 days in EDTA, there were few or no cells giving a peroxidase reaction (Fig. 14).

In old roots of *Allium sativum*, growing in the absence of nutrients in distilled water for 20 days or longer, peroxidase reactions in the protoderm were found to be weak and there were cells that gave no reaction at all. These roots were only 1/3 the diameter of the young roots and their daily rate of growth was about 10% of the normal. In this senescent decline in growth there was a correlated loss of peroxidase.

Relation of Phenols and Naphthols to Decline of Peroxidase Activity

Peroxidase reactions of the older epidermal cells were obtained primarily in the walls of the cells and not in the protoplasts. At the point in maturation where protoplasts of the epidermis and endodermis gave weak peroxidase reactions phenols and naphthols were detected by converting them to their highly colored nitroso forms. In older cells peroxidase reactions appeared to have been swept out of the cell and into the wall at the end of the cell division phase. The end of the division phase was marked by the development of optically detectible phenols, naphthols, and anthrols (23). These substances were identified by converting them to their highly colored nitroso forms (by means of nitrous acid followed by mild alkali to give highly colored polymerized substances). Red polymerization products of nitrosophenols

were obtained only in older portions of histogens at the end of their division phase. At this locus in the root peroxidase reactions were weak, took place largely in walls only, and went over to the second step in oxidation (benzidine brown) with only a momentary holding of the end product of the first step in oxidation (benzidine blue). In older cells containing phenols the oxidation of peroxidase indicators occurred without use of hydrogen peroxide; this indicated the presence of phenolase, in younger cells closer to the meristem tip no phenolase activity could be observed. When phenols or phenolases were detectible peroxidase reactions were weak in the cytoplasm, largely confined to the cell wall.

Coupling of Quinonediimines with Basophilic Substances

A positive test for peroxidase in a given protoplast was found to be a result of the localized activation of peroxidase and the exposure of basophilic substances adsorbing oxidized quinonediimines from the oxidation of phenols by peroxidase. Quinones were strongly adsorbed at the time of their formation and could be removed from the centers of adsorption only on prolonged treatment with alcohol and the usual solvents for basic dyes. It was necessary in some trials to use acid-alcohol to remove quinones from basophilic substances in the cytoplasm. Quinones formed by autoxidation of the parent substances, or by oxidation with extracted peroxidase and hydrogen peroxide, were introduced to tissues under test and a poorly defined localization of quinone staining was obtained in the sites for peroxidase. Coincidence of adsorption of basic end products of peroxidase reaction was further studied by the adsorption of basic stains.

Metachromatic staining with thionin and methylene blue revealed a general coincidence of peroxidase and basophilia. Cells of the protoderm gave the expected "normal" blue color in the cytoplasm indicating the presence of acid substances, cells of the cortex stained lavendar or purple as in a "metachromatic" shift. The cytoplasm of each cell showing strong peroxidase reactions also gave reactions for basophilia. Adsorption of quinones in these centers was found to inhibit peroxidase. The quinonediimine of *p*-phenylenediamine was adsorbed from either alcoholic or water solution and there was a concomitant decline in peroxidase.

Basophilia in specific loci was modified by digestion with ribonuclease by the methods of Brachet (1). Every cell in a root section had an increase of basophilia, but also of peroxidase activity, after mild digestion with ribonuclease. Mild lysis by ribonuclease for 1 hour released peroxidase activity in all cells and increased the basophilia in all cells. Initial lysis by ribonuclease may have given merely a more ready access of reagents to peroxidase and increased the quantity of base adsorbing substances. Prolonged digestion and proteolysis by ribonuclease induced a peroxidase reaction in tissues that normally gave no reaction for peroxidase and resulted in a loss of peroxidase reaction in primordia and histogens commonly having a pronounced peroxidase in the cytoplasm. Extensive digestion lasting for 24 hours resulted in a complete loss of peroxidase and basophilia from the cytoplasm of the

proepidermis and at the same time peroxidase reactions were obtained only in the walls of these cells. Ribonuclease digestion changed the peroxidase reaction from one of division phase to one of old age (Fig. 15). Reactions for phenolase were obtained in these young cells converted by ribonuclease to "old" cells. Ribonuclease digestion in general gave the appearance of removing basophilic substances, freeing the haem from the cytoplasmic peroxidase, promoting adsorption of the released haem to the cell walls, and releasing or activating phenolase systems. All of these phenomena are associated with the sequence of enzyme differentiation in the development of the endodermis and the xylem (24). But the results presented here must be interpreted from complex variables which have yet to be dissected; there is however the conclusion that induced proteolysis gives rise to a sequence of events similar to those of normal differentiation and maturation.

Discussion

Histochemical studies reported here revealed several general phenomena. (*a*) Peroxidase is detectible either in advance of cell division or accompanying cell division in the proepidermis, proendodermis, protophloem, the primordial centers for the origin of buds and roots, wound meristems, the developing nucellus, and embryo sac. (*b*) Peroxidase loss or inhibition is associated with the cessation of cell division. (*c*) Decline of reactive peroxidase takes place in every tissue with the exception of the phloem. (*d*) Decline of peroxidase activity at the end of the division phase is related to the increase of phenols, naphthols, and anthrols and the development of phenolase. (*e*) In the primordial zone peroxidase is cytoplasmic and submicroscopic, in older tissue it is in the mitochondria, but in the mature cells the haem prosthetic group is apparently separated from the protein and becomes lodged in the cell wall. (*f*) Adsorption of quinonediimines, produced by the oxidation of phenols by peroxidase, takes place with nucleic acids or other basophilic protoplasmic substances. (*g*) Peroxidase is general in distribution but open to reaction primarily in basophilic cells. (*h*) The peroxidase system of the phloem is continuous and unbroken and connects all meristems with the oldest tissues and with all organs.

Reported decline of peroxidase activity from meristem to older cells (13, 7) is in general correct for all tissues, but there is one exception. Peroxidase does not decline in the phloem. The absence of phenols in the normal viable phloem may be a corollary of the presence of peroxidase. Inhibition of peroxidase by phenols is an established fact (8) and phenol inhibition of peroxidase probably takes place in many tissues (17, 16), but it is significant that there is no evidence for phenols in the viable phloem at any age. Diazonium coupling tests and other tests for phenolic compounds in the young or mature phloem elements are inconclusive. Diazotized safranin couples with sieve tube contents strongly and in HCl will not decolorize in 24 hours whereas ordinary safranin basic dye is removed in 2 minutes. Whether there are phenols in the phloem is not clear, but it is quite clear that phenol

inhibition of peroxidase does not obtain in the phloem. Peroxidase is apparently a stable porphyrin inclusion within the glucose complex of the phloem.

Peroxidase is continuous and reactive throughout all phases of the development of the phloem. Peroxidase of the adult phloem is in unbroken cytoplasmic contact in sieve tubes and companion cells with the youngest dividing elements of the phloem mother cells. In the sieve tubes respiratory activity is weak and the glycolytic system is rudimentary (25), but the continuous peroxidase system of the phloem connects all meristems with adult tissue and constitutes a continuous redox system in the phloem. A redox cycle could be effected by peroxidase, with oxidizable substances of different redox potential levels, in old and young tissue. Activation of primordia and of histogens could thus be initiated by adult cells at a distance. This activation may be by a mechanism of reaction dependent upon diffusion, but a continuous system of peroxidase could catalyze oxidation in primordia by a redox cycle in which the initation of change in potential originates in old tissue and catalyzes critical oxidation essential to the direction and rate of cell division in primordia. Peroxidase may catalyze the reduction of hydrogen acceptors essential to cell division; cells containing oxidizable material, protected from oxidation in dormancy, may be evocated to division by the activation of the peroxidase system.

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EXPLANATION OF FIGURES

FIG. 1. Localized oxidation and adsorption of 2,6-dichlorophenolindophenol in the proepidermis of the root meristem of the wax-pod bean. ×360.

FIG. 2. Peroxidase oxidation of benzidine in the nucellus and embryo sac of *Zea mays*; to demonstrate the adsorption of benzidine blue in the egg apparatus and nucellar wall around the embryo sac, and the negative reaction and adsorption in the antipodal cells of the embryo sac. ×180.

FIG. 3. Localization of peroxidase in discrete loci defining the form of the metaxylem and its surrounding companion cells. ×290.

FIG. 4. Apical meristem of *Zea mays* from the early vegetative phase of the seedling to show localized peroxidase reaction in a young leaf initial and in the axil of an older leaf initial. ×80.

FIG. 5. Peroxidase reaction in the flowering meristem of *Zea mays* showing peroxidase activity in whorls of cells that have a spiral pattern in the meristem in advance of floral initials that develop later in the same pattern. ×40.

FIG. 6. Enlarged view of the left side of the meristem shown in Fig. 4. ×120.

FIG. 7. Root meristem of *Zea mays* at the first moment of peroxidase activity in the oxidation of benzidine in the root cap initials, the proepidermis and the protophloem histogens. ×120.

FIG. 8. Whole mount unsectioned apical meristem of *Zea mays* showing localized peroxidase of benzidine and localized adsorption of benzidine blue in the axil of a leaf initial. ×120.

FIG. 9. Enlarged view of the axillary region of the meristem shown in Fig. 8. ×290.

FIG. 10. Cross section of living root of *Zea mays* sectioned on the freezing microtome to show the localization of peroxidase in the protophloem. ×120.

FIG. 11. Fresh freehand section of a bean root meristem to demonstrate peroxidase localization in the protoderm and hypodermal cells of the cortex and the negative reaction in the two overlying layers of the root cap. Oxidized leuco base of 2,6-dichlorophenolindophenol was adsorbed so strongly by these cells as to allow clearing in alcohol, benzene, and cedar oil and a final mounting in balsam. ×290.

FIG. 12. Localization of peroxidase in the proepidermis (protoderm) of the root meristem of *Allium sativum* to show the concentrated oxidation of reduced 2,6-D by peroxidase. ×290.

FIG. 13. Inhibition of peroxidase activity by EDTA at a concentration of 10^{-5} *M*. ×290.

FIG. 14. Inhibition of both cell division and peroxidase activity by EDTA at a concentration of 10^{-2} *M*. ×290.

FIG. 15. Peroxidase activity confined to the walls of the protoderm in a root section of *Allium sativum* after incubation with ribonuclease. Ribonuclease catalyzes a digestion localizing peroxidase exactly as it appears in the late phase of differentiation of this tissue. ×120.

PLATE I

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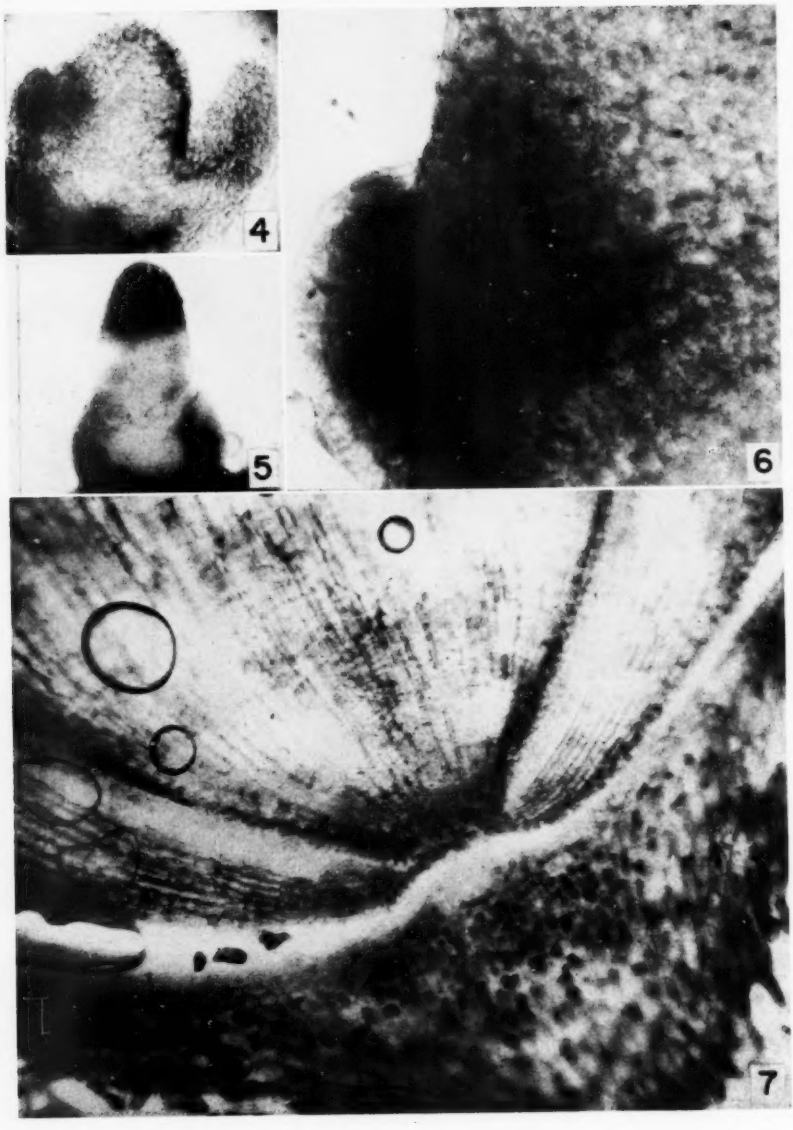
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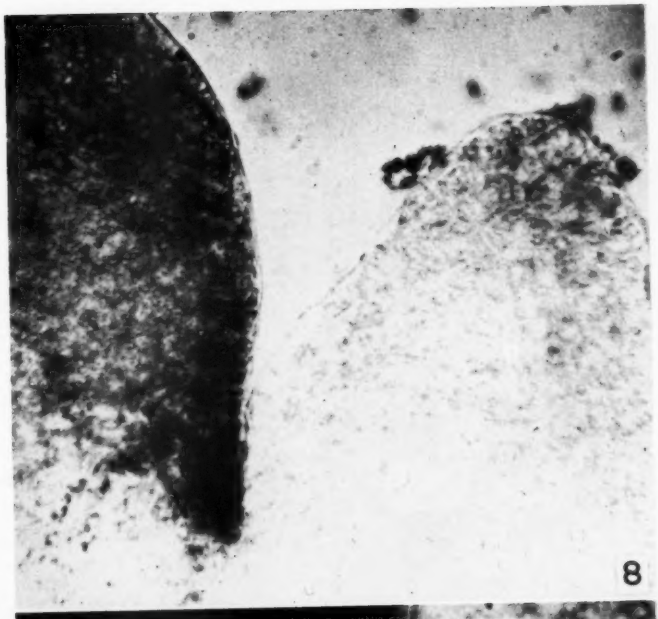
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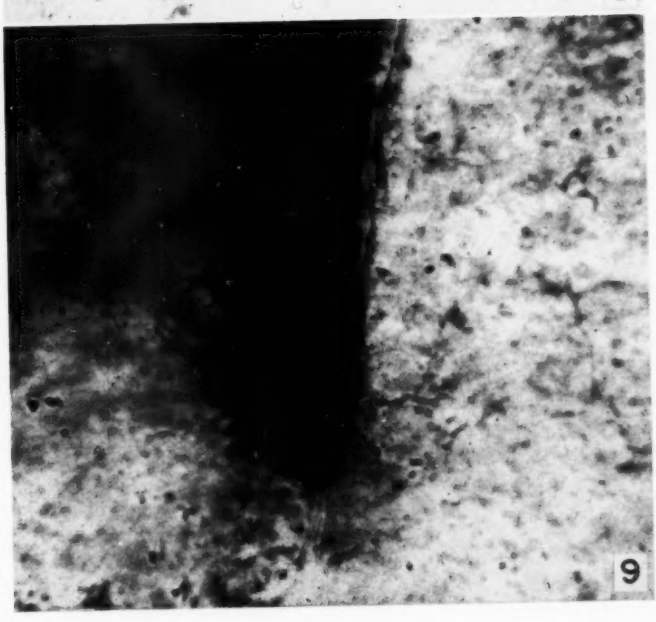
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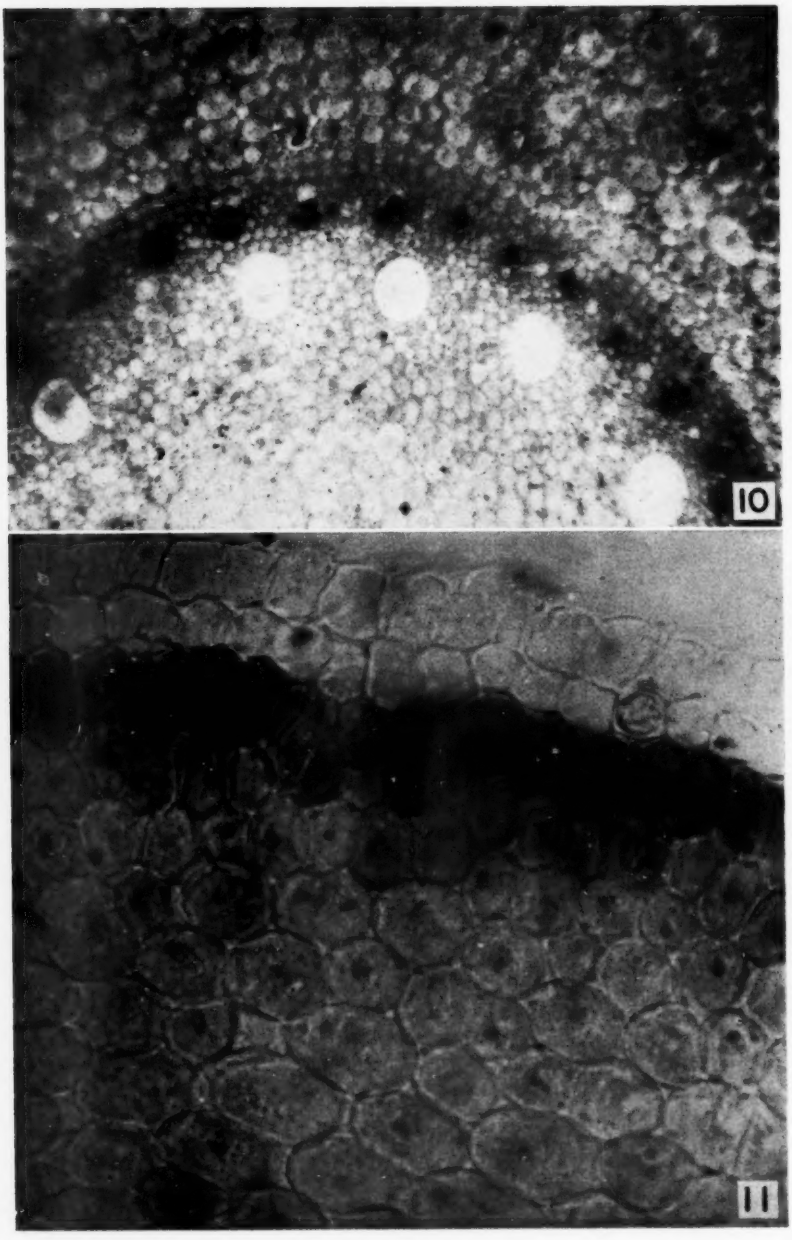

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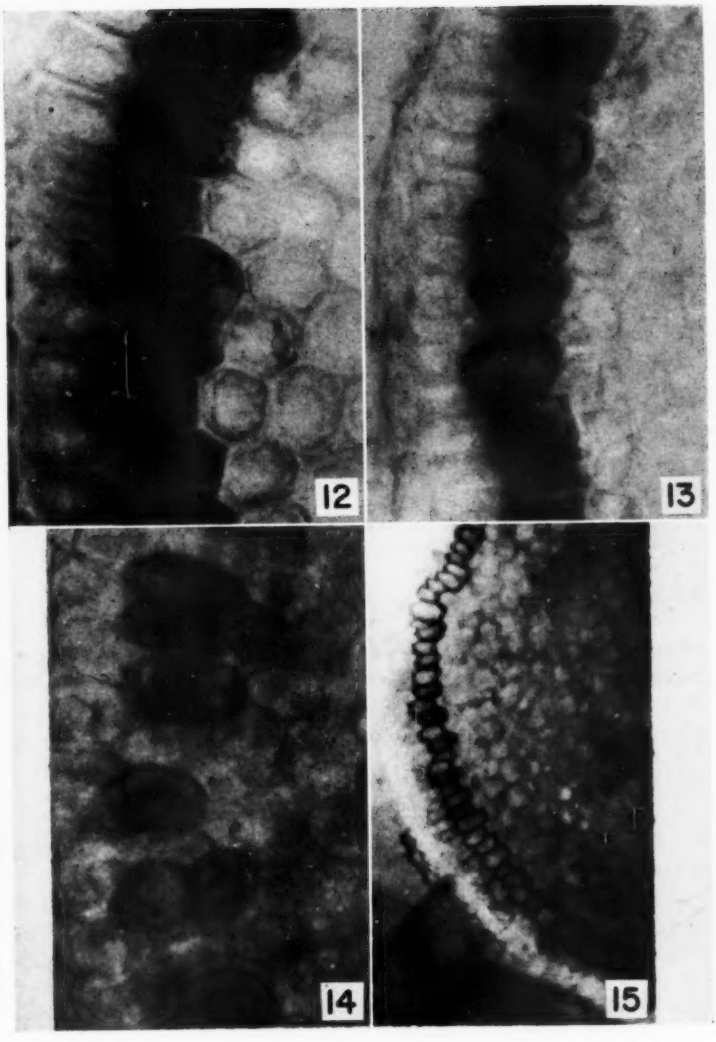
PLATE V



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THE PEACH REPLANT PROBLEM IN ONTARIO

VII. THE PATHOGENICITY OF PRATYLENCHUS PENETRANS (COBB, 1917) FILIP. & STEK. 1941[1]

W. B. MOUNTAIN AND Z. A. PATRICK[2]

Abstract

Experiments were carried out to determine the role of *Pratylenchus penetrans* (Cobb, 1917) Filip. & Stek. 1941 in the peach replant problem. It is shown that this nematode is a true plant pathogen since it invades and causes necrosis of peach roots in the absence of bacteria and fungi. Necrosis of the root tissue occurs rapidly and appears to take place in advance of the invading nematode. The main mechanism involved in the formation of the lesion is the production of phytotoxic substances through hydrolysis of the cyanophoric β-glucoside, amygdalin. *P. penetrans* is capable of hydrolyzing this substance in vitro. It is concluded that, although *P. penetrans* is the most important primary parasite and a true plant pathogen, its main role in peach replant failure appears to be its ability to incite root degeneration by providing extensive infection courts for soil microorganisms.

Introduction

In previous papers of this series Mountain and Boyce (10, 11) have shown that in terms of distribution, occurrence, and severity of the disease in Ontario, there is a close and constant association between peach replant failure and *Pratylenchus penetrans*. They concluded, however, that the precise role of this nematode in the disease complex could only be determined by laboratory study. Patrick (12) postulated that regardless of the causal organism involved the basic mechanism of lesion formation in peach roots is likely to be the production of phytotoxic substances principally through the hydrolysis of the cyanophoric β-glucoside, amygdalin.

The purpose of the present studies was to evaluate further the role of *P. penetrans* in peach replant failure by determining whether the nematode can produce necrosis of peach roots in the absence of other organisms and by attempting to clarify the mechanisms involved in the formation of the lesion.

Materials and Methods

Method of Obtaining Nematodes Free of Bacteria and Fungi

In most of these studies *P. penetrans* originally obtained from roots of peach were used, but in some of the tests the nematodes were obtained from tobacco and vegetables. They were maintained and multiplied in the greenhouse mostly in the roots of vetch (*Vicia villosa*). Occasionally, *P. penetrans* maintained in the roots of rye were also used. Periodic examination ensured that *P. penetrans* was the only plant parasite present.

The procedure used for obtaining large numbers of *P. penetrans* free of bacteria and fungi is not entirely original and a number of other workers, particularly Drs. B. F. Lownsbery (8) and R. S. Pitcher (personal communications),

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have contributed to the development of this method. The main steps of the method are listed below.

Eight-inch glass funnels, each fitted with a short piece of rubber tubing and a stopcock, were washed in a 1:1000 solution of mercuric chloride and placed in a stand. The funnels were filled with a bactericide–fungicide solution. The most effective solution developed to date by trial and error and found to be nonnematocidal has the following composition: ethoxyethylmercury chloride 4 p.p.m., dihydrostreptomycin sulphate 1000 p.p.m. In some instances, especially those in which nematode-infested rye roots were used, a few bacterial contaminants were still obtained after treatment with the above solution. Most of these were eliminated by the addition to the solution of 30 p.p.m. of aureomycin in HCl.

Stainless steel wire rings made to fit the funnel about one inch from the top were covered with fine muslin. The screens so formed were first wrapped separately in paper and sterilized by autoclaving. One screen was then placed in the top of each funnel and the solution drawn off at the bottom until the cloth was just covered by the fluid. The nematode-infested vetch or rye roots were removed from the soil in the greenhouse and washed thoroughly under a stream of water to remove foreign matter and dead roots. The roots were cut up in approximately 5-mm sections and spread carefully on the screen, care being taken that no root particles fell below the screen.

At intervals of 24 hours over a period of approximately one week, the stem of the funnel was washed with 1:1000 mercuric chloride solution and approximately 30 ml of the liquid containing the nematodes drawn off from the bottom of the funnel into a sterile test tube. The tube was allowed to stand for 1 hour, by which time most of the nematodes had settled to the bottom. The top 20 ml of each tube were decanted and the remainder was poured into a sterile Syracuse watch glass and examined under the microscope. All nematodes, other than *P. penetrans*, were drawn off with a sterile pipette and discarded. The remaining nematodes were counted and returned to the tube. They were then allowed to remain in the sterilizing fluid for an additional hour.

By means of settling and decanting, the nematodes were washed twice in well-aerated, sterile, distilled water. By using a sterile pipette an aliquot of the inoculum, containing a known number of nematodes, was poured into a tube of thioglycollate broth, as outlined below, to test for the presence of fungal and bacterial contaminants.

Normally, each funnel yielded about 1000 *P. penetrans* per day. In approximately 60% of the tests this method gave active, infective nematodes free of culturable bacteria or fungi.

Method of Growing Peach Seedlings under Controlled Conditions

To study the effect of various population levels of *P. penetrans* upon the growth of peach seedlings under constant and optimum growth conditions for the host, experiments were carried out in a growth room in which light and temperature were controlled. To reduce as much as possible the variability

due to the activity of soil microflora, peach seedlings were grown in washed quartz sand watered with a nutrient solution which was almost entirely inorganic. Details of the method follow.

Three-inch unglazed clay pots were used, the drain holes of which were plugged with a cork. The pots were filled with quartz sand (Flint-Shot grade) and sterilized by autoclaving. Peach seeds were germinated in sterile compost soil, and seedlings were selected that were approximately one inch high, had healthy roots, and were of uniform appearance. The roots were washed thoroughly and the seedlings were planted one to each pot. A known number of microbiologically sterile *P. penetrans*, obtained by the method described above, was added to each pot and the sand was then watered with a nutrient solution. The nutrient solution used was essentially that described by Hoagland and Arnon (7), iron citrate being added at triple the recommended rate. To prevent the growth of algae on the surface of the sand, the top of each pot was covered with a circular aluminum disk with a hole cut in the center for the plant. Preliminary experiments showed that seedlings grown in the nutrient solution appeared normal and the rate of growth was at least equal to that of peach seedlings growing in sterile compost soil for the same 12-week period.

After the seedlings were planted and inoculated, each pot was placed within a 5-in. pot and the space between filled with vermiculite which was kept moist during the experiment. Four of these double pots were then placed in a plastic tray approximately 12 in. × 13 in. × 6 in. deep and the remaining space in the tray filled with sand which was kept moist (Fig. 1). The trays were then placed in a soil temperature tank adjusted so that the soil temperature remained constant at 65° F while the air temperature of the room was maintained at 72° F during illumination and 65° F during darkness. The plants received 1000 ft-c of artificial illumination during a 16-hour day. Nutrient solution was added to the quartz sand at intervals as required. Results were expressed as the increase in growth of the seedlings measured in millimeters.

Method of Obtaining Sterile Peach Roots

Peach seeds, obtained from the variety Lovell, which had been afterripened for 60 to 100 days at 4° C were used. Only plump seeds with intact seed coats which showed no microbial damage were selected. The seeds were scrubbed with a stiff toothbrush under running water for approximately five minutes. They were then placed in a 50% solution of ethyl alcohol for 1 minute, a 1:1000 solution of mercuric chloride for 3 minutes, and finally in a solution consisting of 0.25% Aretan (a formulation of Bayer Agriculture Ltd., containing 3% ethoxyethylmercury chloride) and 0.25% Arasan (a formulation of E. I. DuPont de Nemour containing 75% thiram) for 5 minutes.

The treated seeds were placed in germination chambers prepared as follows. Two layers of filter paper were placed in the bottom of Petri dishes, the walls of which were lined with absorbent cotton. The dishes were sterilized by autoclaving. Four of the treated peach seeds were then placed in each dish

together with 25 ml of sterile water. The dishes were then kept in the dark at 65° F until the seeds germinated. When the developing radicles were approximately 10 mm long, the seeds were examined carefully and any showing evidence of fungal or bacterial growth were discarded. Even with this method, 75 to 85% of the seeds eventually showed bacterial or fungal growth. In most instances, however, contamination was confined to the cotyledons and sections of roots plated out at intervals on various media were free of microorganisms.

Germinated peach seeds were then transferred individually to culture tubes which were prepared as follows. Test tubes (25×200 mm) were filled with quartz sand to a height of 80 mm, and 10 cc of distilled water was added. The tubes were plugged with nonabsorbent cotton autoclaved for 40 minutes at 15lb pressure. After the tubes had cooled, one germinated seed was carefully pushed into the sand in each tube. The tubes were placed in the dark at 65° F until a well-developed root system had formed (Fig. 2). Usually much of the root system developed immediately beneath the inner wall of the tube and thus could be observed readily. As soon as a well-developed root system had formed, known populations of nematodes which had previously been freed of bacteria and fungi by the method described above were added to each tube aseptically. This was done with a sterile pipette and care was taken to place the nematode inoculum close to the peach roots.

At the end of an experiment, the outer surface of each test tube was washed in a 1:1000 solution of mercuric chloride; the tube was then wrapped in a sterile towel and broken open aseptically. Lesioned areas on the roots were removed and plated out on various nutrient media to test for the presence of microorganisms.

Culture Media Used

The various culture media used in the experiments and the main purpose for which they were employed are listed below.

To determine whether the nematode inoculum was microbiologically sterile after it had been treated in the manner described, a portion of the inoculum was added to tubes of N.I.H. thioglycollate broth (3) as suggested by Lownsbery and Lownsbery (8). The inoculated tubes were incubated for a 3-week period and only data from experiments in which the nematode inoculum was free of bacteria and fungi were used. In many instances 1% agar was added to the above medium and the treated nematode sample was also plated out on this medium.

To test for the presence of microorganisms in peach roots after they had been inoculated with *P. penetrans* the roots were plated out on the following media:

Medium 1. N.I.H. thioglycollate broth + 1% agar.

Medium 2. Bacto-peptone 5 g, beef extract 3 g, dextrose 3 g, yeast extract 1 g, agar 15 g, water 1000 ml; pH 6.6.

Medium 3. Bacto-yeast extract 10 g, agar 15 g, water 1000 ml; pH 6.6.

Medium 4. Bacto brain heart infusion 35 g, yeast extract 2 g, soil extract 100 ml, agar 15 g, water 1000 ml; pH 5.8.

Medium 5. Bacto-peptone 2 g, beef extract 2 g, soil extract 100 ml, dextrose 2 g, yeast extract 2 g, skim milk powder 50 g, sodium chloride 2 g, potassium phosphate 2 g, agar 12 g, water 1000 ml; pH 6.5.

All media were prepared in the conventional manner and autoclaved for 15 minutes at 15 lb pressure. The soil extract was prepared according to the method found in Fred and Waksman's laboratory manual (5).

To determine the ability of *P. penetrans* to hydrolyze amygdalin in vitro, living, microbiologically sterile nematodes were added to the following media:

Medium 6. Amygdalin 50 g, peptone 20 g, yeast extract 5 g, potassium phosphate 2 g, agar 10 g, water 1000 ml; pH 5.5.

Medium 7. Amygdalin 50 g, peptone 10 g, potassium phosphate 1 g, agar 15 g, water 1000 ml; pH 5.0.

Medium 8. Amygdalin 50 g, potassium phosphate 2 g, agar 15 g, water 1000 ml; pH 5.0.

The media were prepared in the conventional manner, placed in 20-ml quantities in test tubes, and autoclaved for 15 minutes at 15 lb pressure. After the media cooled to approximately 45° C, 1 ml of sterile water containing known numbers of microbiologically sterile *P. penetrans* was added to each tube, mixed, and poured into sterile Petri dishes. After the agar had solidified, each plate was wrapped separately with aluminum foil and incubated at room temperature of approximately 25° C. The plates were examined every 5 days and those showing bacterial or fungal growth were discarded. To determine whether amygdalin was hydrolyzed by *P. penetrans*, a test for HCN was carried out using the "sodium carbonate picric acid paper test". The sodium carbonate picric acid paper (hereafter referred to as Na picrate paper) was prepared according to the method described in the Official Methods of Analysis of the Association of Official Agricultural Chemists (2). If HCN is present, the yellow Na picrate paper gradually turns orange then brick red. The test is delicate and the rapidity of change in color depends upon the quantity of free HCN present. The moist Na picrate paper was placed over, but not touching, the agar in the Petri dish. The dish was covered and the color change was noted after a $\frac{1}{2}$- and 1-hour interval. In some instances the nematodes were added to 10-ml quantities of the above media in screw-cap test tubes and tests for HCN were carried out by suspending strips of Na picrate paper near the top of the test tube.

Observations and Results

The Effect of Pratylenchus penetrans upon Growth of Peach Seedlings

A number of preliminary experiments carried out in the greenhouse in which peach seedlings were planted in sterilized soil inoculated with known numbers of *P. penetrans* indicated that after penetration by the nematode the roots became necrotic and the seedlings stunted. However, these experiments did not completely define the role of the nematode in the disease complex since bacteria and some fungi were invariably present in the nematode inoculum. Following the development of a method of obtaining microbiologically sterile

P. penetrans, attempts were made to study the effect of the axenic nematode inoculum on the growth of peach seedlings. The experiment was carried out as follows:

By the method described previously, 48 uniform peach seedlings were planted in quartz sand in double pots placed in plastic trays (Fig. 1). The experiment was set up in the growth room as described. The nematode inoculum consisted of known numbers of *P. penetrans* obtained from vetch roots in the described manner and made up into a series of aliquots each in 10 ml of sterile water. These series consisted of either 200, 400, or 800 *P. penetrans* per aliquot and one such aliquot was poured over the surface of each pot of quartz sand selected at random. An identical series, except that no nematodes were added, served as controls. The pots were labeled and the height of each seedling measured.

At the end of 14 days the leaves of the plants in the series that received 800 *P. penetrans* were chlorotic and the plants noticeably stunted. Four weeks after inoculation, the total linear growth of each seedling was measured and the average net increase in growth calculated. These data are summarized in Table I.

TABLE I

The effect of *P. penetrans* upon the growth of peach seedlings

Number of nematodes added, per pot	Net growth increase, mm
0	459
200	276
400	180
800	117
L.S.D. 5%	59

It is apparent from Table I that *P. penetrans* reduced the growth of peach seedlings under otherwise optimum growth conditions for the host and that the amount of stunting is roughly proportional to the concentration of the nematode inoculum. Marked differences were also noted in the appearance of the roots. Whereas the roots of peach seedlings in the series that had received the nematode inoculum were discolored and necrotic those in the control series were white and apparently healthy. It should be stressed, however, that in this experiment the peach seedlings used were not microbiologically sterile.

The Production of Lesions on Peach Roots by Microbiologically Sterile Pratylenchus penetrans

In these experiments attempts were made to determine whether *P. penetrans* can invade and, through its parasitic activities, cause necrosis of peach roots in the absence of bacteria and fungi. For this purpose known numbers of microbiologically sterile *P. penetrans* were added to culture tubes con-

taining a sterile, well-developed peach root system as described above. The attack of the nematodes upon the peach roots growing immediately under the glass was observed by means of a dissecting microscope at a magnification of 50.

These experiments were repeated a number of times with varying amounts of inoculum that contained from 350 to 3500 nematodes. Since the sequence of lesion formation was essentially identical in all cases, a typical sequence of events is described below.

After inoculation, the nematodes appeared to move quite rapidly toward the nearby peach roots, in some cases traveling up to 7 mm through the sand in approximately four hours. The nematodes were then observed clustered along the root surface, presumably in the process of penetrating the epidermal cells. Within 90 minutes after a single preadult or adult nematode reached a peach root, a very small discolored area comprising a few epidermal cells of the root was observed under the microscope. The affected cells were orange-brown in color in marked contrast to the normal glistening white root tissue (Figs. 2, 7). By the end of 3 hours the discolored area was noticeably larger surrounding the head of the nematode. By the end of 9 hours the nematode was partly imbedded in the root tissue and the discolored area by this time had spread into a distinct tan-colored streak or fleck, clearly visible to the naked eye. By the end of 24 hours many of these flecks had coalesced so that a distinct lesion was clearly visible. The lesioned areas continued to enlarge and by the end of 96 hours discolored and apparently necrotic areas up to 2 cm in length could be seen on the roots (Fig. 4). The experiments were allowed to run from a few hours to a month after inoculation.

At various intervals from 9 hours to 34 days after the addition of the nematodes, the tubes were broken open aseptically and lesioned portions of the roots plated out on the various nutrient media described previously. The tissue was either transferred directly to plates of nutrient agar or first washed in sterile water. In no instance, however, was any chemical treatment used to surface-sterilize the roots before plating. Other lesioned areas were stained with lactophenol acid fuchsin and cleared for microscopic examination.

Microscopic examination of the incipient lesion, approximately nine hours after the nematode began feeding, invariably showed at least one nematode partly buried in the tissue. The cortical cells surrounding the nematode were definitely necrotic and contained shrunken, granular cytoplasm. At this stage too, the necrotic area extended for a number of cells beyond the limit of nematode penetration and this suggests the occurrence of some action in advance. Microscopic examination of a well-developed lesion, approximately one week old, showed as many as 37 adult and larval nematodes and 50 eggs within a lesioned area of approximately two centimeters in length (Fig. 3). Older lesioned areas 4 to 5 weeks old contained large numbers of *P. penetrans* in all stages of development.

Isolations made from necrotic areas on the roots at various intervals from 9 hours to 34 days after the addition of *P. penetrans* on a wide variety of

culture media failed to show the presence of any fungi or bacteria in these tissues. As a check on the isolation technique, duplicate experiments were carried out in which a bacterial culture, originally isolated from *P. penetrans* obtained from peach roots, was added to the nematode suspension before inoculation. In every case, this same bacterial isolate developed on the nutrient agar plates within 2 days after plating the necrotic tissue. In all of the tests carried out to date, bacteria were isolated from the root lesions only in cases where they had been added deliberately to the nematode inoculum.

Since in these experiments it has been shown that *P. penetrans* can invade, colonize, and kill peach root tissue in the absence of other organisms, it can be concluded that the nematode is a true plant pathogen.

Evidence for Hydrolysis of Amygdalin by P. penetrans

A. In Vivo

As indicated in the preceding section, killing of peach root cells occurs rapidly and apparently in advance of the area of penetration thus suggesting that toxic substances are involved. Since HCN and benzaldehyde, which result from hydrolysis of the amygdalin occurring in peach roots (1, 4, 9), might constitute a source of such toxins, experiments were designed in an attempt to determine whether amygdalin was being hydrolyzed in that portion of the root invaded by the nematode.

To determine whether detectable amounts of HCN were being released from nematode-induced lesions, peach seedlings were grown in quartz sand in modified filter tubes (40×180 mm) and inoculated with microbiologically sterile *P. penetrans*. Six of these tubes were placed in a rack and a stream of sterile air was passed slowly through the sand of each tube and into a bubbler containing Na picrate solution (Fig. 5). To serve as a control, six noninoculated tubes were also set up in a rack and aerated as above. Each day, the tubes in both series were aerated separately for approximately 30 minutes. A weak positive test for HCN was obtained at the end of 12 days after inoculation. In this test there were approximately 3500 *P. penetrans* per tube. No test for HCN was obtained in the control. Since only a weak positive test for HCN was obtained it is likely that most of the free HCN produced in the root lesion by the hydrolysis of amygdalin had diffused to adjoining cells and been adsorbed.

At the completion of the experiment, both series were dismantled and it was observed that in the series that received the nematode inoculum the roots were necrotic, whereas in the control series the roots were white and apparently healthy (Figs. 6, 7, 8).

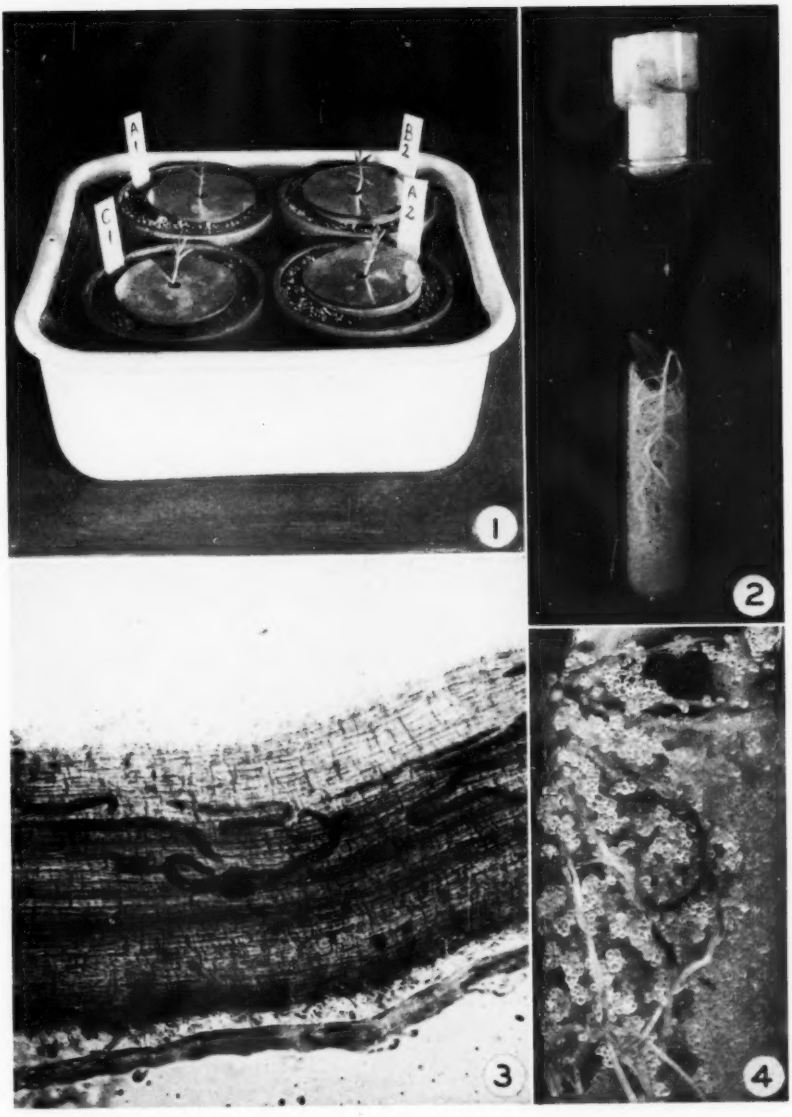
FIG. 1. Peach seedlings growing in quartz sand and nutrient solution used for inoculation studies with *P. penetrans*.

FIG. 2. Peach seedling growing in a test tube under sterile conditions for use in pathogenicity tests with *P. penetrans*.

FIG. 3. Section of a lesion, 24 hours after the addition of microbiologically sterile *P. penetrans*, showing nematodes invading and colonizing peach root tissue.

FIG. 4. Lesions on peach roots produced by microbiologically sterile *P. penetrans*. The photograph was taken 96 hours after the addition of the nematodes.

PLATE I



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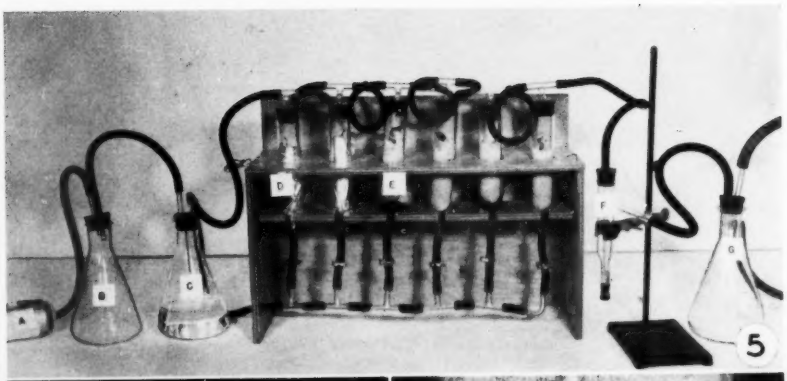

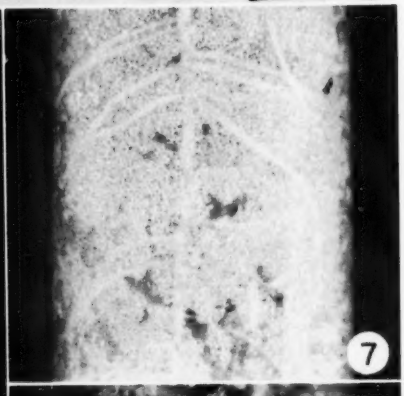

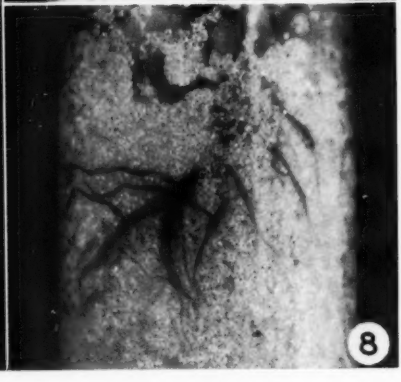

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In additional experiments, further evidence for amygdalin breakdown in the lesioned area was obtained. In these tests the relative amount of amygdalin present in a lesioned area was compared with the amount found in an adjacent healthy area. Culture tubes, each containing a well-developed peach root system, were inoculated with *P. penetrans* as in the preceding section. Three weeks later, when lesions were well developed, the roots were removed and 10 pieces of necrotic root, each approximately 10 mm long, were placed in a test tube and crushed with a glass rod. The test for HCN was carried out in the usual manner by suspending a strip of Na picrate paper over the macerated roots in a corked test tube. After 30 minutes, the color change of the paper was recorded. An exactly similar quantity of healthy root tissue from the same root and often adjacent to the lesioned area was removed and treated in exactly the same manner. In other tests, a commercial preparation of emulsin was added to the crushed root tissues to determine whether any amygdalin remained in the necrotic areas. Conversely, a commercial preparation of amygdalin was added to crushed root tissue to determine whether any of the naturally occurring enzyme was still active in the necrotic areas. The results of all of these tests are summarized in Table II.

TABLE II

Relative amount of HCN produced by peach root tissue obtained from healthy and lesioned areas

Treatment	Relative amount of HCN produced in 30 min by peach root tissue from:	
	Healthy areas	Lesioned areas
Crushing alone	+++*	−
Crushing + emulsin	+++	−
Crushing + amygdalin	+++	+

*The amount of HCN produced as determined by the intensity of red color of the Na picrate paper produced after 30 minutes in the reaction vessel; +++ strong positive test for HCN, + weak positive test, − thin negative test.

It is apparent from these results that, as the amygdalin was not present in the nematode-induced lesion, hydrolysis of the glucoside must have taken place in the affected area. Since the products of such hydrolysis, namely

FIG. 5. Apparatus used to detect the presence of free HCN following the addition of microbiologically sterile *P. penetrans* to peach seedlings. A, "Koby" air filter. B, trap. C, water filter and hydrator. D, filter tube containing a peach seedling inoculated with microbiologically sterile *P. penetrans*. The tubes were wrapped in aluminum foil during the experiment. E, filter tubes with the aluminum foil removed showing the peach seedling growing in quartz sand. F, bubbler containing Na picrate solution used for HCN determination. G, trap leading to vacuum pump.

FIG. 6. Tubes from the control series (left) and the series receiving 3500 *P. penetrans*. Photograph taken 12 days after inoculation.

FIG. 7. Enlargement of the peach root system from the control series showing normal white, glistening roots.

FIG. 8. Enlargement of the peach root system from the inoculated series showing extensive necrosis.

HCN and benzaldehyde, were shown to be toxic to peach root tissue (12), the action in advance described in the preceding section could be due to this reaction.

B. Hydrolysis of Amygdalin by P. penetrans in Vitro

The hydrolysis of amygdalin in the nematode-induced root lesion could occur in at least two ways. *P. penetrans* could so injure peach root cells, either mechanically or chemically, as to bring together the glucoside and its splitting enzyme present in these cells. The nematode also might secrete a β-glucosidase which would hydrolyze the amygdalin directly.

To test the ability of *P. penetrans* to hydrolyze amygdalin, experiments were carried out in which microbiologically sterile populations of *P. penetrans* were added to agar media containing amygdalin. The composition of the media used and techniques followed have been described earlier.

The experiments have been repeated at least 50 times. In each instance the Petri plates were carefully examined for the presence of bacteria and fungi and, where these contaminants were found, the results were not used. In many of these experiments, a positive test for HCN was readily obtained in plates when only *P. penetrans* was present. It was found, however, that a positive test could only be obtained when very active nematodes, which remained alive in the agar medium for at least 25 days, were used. It was also noted that a positive test was more readily obtained when the agar medium contained peptone and yeast extract in addition to amygdalin. A negative HCN test was obtained in the controls with the same media treated in exactly the same manner but to which no living nematodes were added. In the instances where the nematode populations used were not entirely free of bacteria or fungi, it was noted that many of these contaminants, most of which were bacteria, were also capable of hydrolyzing amygdalin readily.

The time required for a positive HCN test to be obtained appeared to depend upon the number of nematodes added to the plate. Thus, with a population of approximately 1000 *P. penetrans*, a detectable amount of HCN was produced in 7 to 10 days, whereas, when approximately 100 *P. penetrans* were added to the same quantity of agar, HCN was detected only after 15 to 25 days.

From these experiments there is little doubt that amygdalin can be hydrolyzed by the secretions from living *P. penetrans*. It is likely that the nematode secretes a β-glucosidase, and attempts are now being made to characterize the enzyme involved. Limited attempts to extract a β-glucosidase directly from nematode tissue by maceration and use of solvents have been unsuccessful to date.

Discussion

The present studies have shown that *P. penetrans* can invade and kill peach root tissue in the absence of bacteria and fungi. Its parasitic activity results in extensive necrosis of peach roots. It has also been demonstrated that populations of *P. penetrans* reduce the growth of peach seedlings in a growth

room under otherwise optimal conditions of growth for the host. These results appear to satisfy the standard rules of proof of pathogenicity and the nematode can thus be designated a true plant pathogen.

However, the role of the nematode in peach replant failure, that is, in mortality of peach trees under natural conditions, must be somewhat more than that of a pathogenic agent. In this study a number of bacterial isolates from *P. penetrans* obtained from peach roots were found to be capable of hydrolyzing amygdalin in vitro and since these bacteria could be carried into root tissue by the nematode, in such cases the latter could be considered as acting as a vector. Then, too, Wensley (14) isolated at least 18 genera and 34 species of fungi from the peach root rhizosphere, and although none of them appeared to be primary parasites, he concluded that many of them could contribute to root degeneration. It has been shown that *P. penetrans* can produce a relatively large necrotic area on peach roots in a matter of a few hours and such a necrotic area could form an ideal infection court for many of the soil bacteria and fungi. In this case the nematode might be acting as an incitant. Therefore it may be postulated that, although *P. penetrans* is a pathogen in its own right, its major role in peach replant failure is in inciting root degeneration through the formation of extensive infection courts.

The mechanism of lesion formation is interesting. At least part of this mechanism appears to be due to toxic substances released through the hydrolysis of amygdalin. The toxic agents are capable of diffusing ahead of the nematode, thus killing peach root cells. The authors have suggested that the hydrolysis of amygdalin can occur in a number of ways. Mechanical and chemical damage to the root cells by the parasitic activities of the nematode could bring together the glucoside and the naturally occurring β-glucosidase, emulsin. Then too, it has been demonstrated in these studies that *P. penetrans* is capable of secreting substances, probably enzymatic in nature, which will also hydrolyze amygdalin. It has not yet been possible to separate these two mechanisms of amygdalin hydrolysis in the root lesion but both of them likely occur and proceed almost simultaneously. Regardless of the mechanism of this hydrolysis, however, it has been shown that amygdalin is completely hydrolyzed in the lesioned area. Furthermore, certain bacteria closely associated with the nematode under natural conditions hydrolyze amygdalin in vitro and are probably carried into the root tissue by the nematode. Unpublished research by Patrick and Wensley has shown that many fungi obtained from the peach root rhizosphere can also hydrolyze amygdalin in vitro. Therefore, production of phytotoxic substances through the hydrolysis of amygdalin appears to be the main mechanism involved in the entire etiological sequence of degeneration of peach roots. Unpublished research by T. B. Harrison of the Harrow Experimental Station and W. B. Mountain support the preceding postulation. They found that seedlings of the variety Shalil supported high root populations of *P. penetrans* without discernible stunting, whereas seedlings of the variety Lovell grown under identical conditions contained smaller root populations of the nematode and were markedly

stunted. Since Ward and Durkee (13) showed that roots of Shalil contain considerably less amygdalin than the roots of Lovell, it is possible that tolerance of a particular root stock to nematode attack and subsequent root degeneration may depend upon the amount of amygdalin present in that root system.

It might be postulated, therefore, that peach replant failure is basically a root-rot disease in which the root lesion nematode *P. penetrans* is the most important primary parasite. The nematode acts both as a pathogen and as an incitant and the stages in the etiology of the root rot after nematode penetration are likely quite nonspecific and unpredictable and involve a number of soil fungi and bacteria. Superimposed upon the pathology of root necrosis are the effects of phytotoxic substances that result from breakdown of peach root residues of the former orchard as postulated by Patrick (12). However, root degeneration does not necessarily result in the death of the tree and it is at this stage, as shown by Harrison (6), where such environmental factors as soil moisture, nutritive levels, weed competition, and quality of nursery stock determine whether the reduced root system is capable of maintaining the vital requirements of the tree.

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COMPARATIVE EFFECTS OF 3-INDOLYLBUTYRIC ACID, 2,3,5-TRI-IODOBENZOIC ACID, AND β-NAPHTHOXYACETIC ACID ON FLOWER GROWTH AND FLOWER WEIGHT OF THE CAULIFLOWER (BRASSICA OLERACEA)[1]

B. C. Das

Abstract

Three synthetic growth-promoting substances were administered to cauliflower plants (*Brassica oleracea*) to determine their effect on flower weight and flower growth. A Youden square experimental design was employed to set up treatment groups for all possible combinations of three substances at four concentration levels and three frequencies of application, and an untreated control group. The substances employed were 3-indolylbutyric acid (IBA), 2,3,5-triiodobenzoic acid (TIBA), and β-naphthoxyacetic acid (NOXA); concentrations used were 0.01, 0.2, 4.0, and 80.0 parts per million; and frequencies adopted were 2, 3, and 6 times in a 12-day period. Final flower weights were analyzed by an analysis of variance which showed that the treatments differed significantly ($P < .01$). Treatments with NOXA and IBA produced flowers weighing significantly more than those of the control group. Flower growth was characterized by a rapid initial rise, which subsequently levelled off in the control group. For treated groups, the initial rise continued throughout the flowering period. The results suggest that the use of certain synthetic growth-promoting substances may be feasible commercially for enhanced production of cauliflower crops.

Introduction

The study of growth-promoting substances in higher plants has received an increasing amount of research attention since the discovery of the auxins, and their role in the control of plant growth, in 1910. It has been demonstrated that at lower concentrations the auxins stimulate growth, e.g., promote root elongation, and the growth of buds and stems, while at higher concentrations they inhibit growth (2). Following the outline of the general functions of the naturally occurring auxins, interest turned toward synthetic compounds producing similar effects. These synthetic growth-promoting substances have been found to be useful commercially in the control of the growth of some economically important fruits, and are employed to induce fruit set, increase fruit size, hasten maturity, and reduce fruit drop. Among the fruits commercially treated are apples, citrus fruits, figs, grapes, peaches, pears, and tomatoes (3). To explore further the feasibility of using synthetic growth-promoting substances in the production of commercial vegetable crops, the present investigation was undertaken, with particular reference to the cauliflower (*Brassica oleracea*).

While the long-range purpose of this type of investigation would be to develop commercially useful treatments, it would be desirable first to explore the effects of several synthetic growth-promoting substances at different concentration levels. With this end in view, the present investigation employed

[1]Manuscript received in original form November 25, 1958, and, as revised, January 12, 1959. Contribution from the Biometry Research Unit, Indian Statistical Institute, 203, Barrackpore Trunk Road, Calcutta 35, India.

three compounds: 3-indolylbutyric acid (IBA), 2,3,5-tri-iodobenzoic acid (TIBA), and β-naphthoxyacetic acid (NOXA) at four concentration levels, ranging from 0.01 to 80 parts per million (p.p.m.). Data were collected on flower growth, in terms of the diameter of the developing flower, and the final flower weight.

Design of the Experiment

Three synthetic growth-promoting substances were investigated experimentally, each at four concentrations and three frequencies. The compounds were NOXA, IBA, and TIBA. The concentrations employed were 0.01, 0.2, 4.0, and 80.0 p.p.m. Application of the compounds took place during a 12-day period at three frequencies: 2 times or every 6th day, 3 times or every 4th day, and 6 times or every 2nd day. A Youden square design was chosen to permit the number of treatments required within the limits of the experimental field plot available, and to control the possible effect of a fertility trend in two directions (1). Taking all possible combinations of the three compounds at four concentration levels and three frequency levels, 36 treatments were defined. With the addition of a control or untreated group, there were 37 treatments. The identification of these treatments is given in the first four columns of Table II. The parameters of the Youden square were $v = b = 37$ (i.e., 37 rows to permit 37 treatments), $r = k = 9$ (i.e., 9 columns or replications of each treatment), and $\lambda = 2$ (1, Plan 13.15). Treatments were assigned according to a randomization scheme.

Materials and Methods

A recognized type of cauliflower seeds (*Brassica oleracea*), Snowball, an early variety[2] was used in this experiment. Prior to planting the seeds, a 6-ft-square seedbed was treated with 10 lb of oil cake, 10 lb of leaf compost, and 4 lb of ammonium sulphate. The seeds were broadcast on the seedbed September 22, 1957. The oil cake was composed of the solid residue obtained after extracting the oil from mustard seeds. It is a common fertilizer in the region where this experiment was conducted.

The experimental field plot was 73 ft 8 in. by 22 ft 4 in. Fifteen days before transplanting the young cauliflower plants, oil cake was placed in the soil for decomposition. One half pound of oil cake was placed in the site for each plant. When the plants were 1 month old, they were transplanted to the field plot. A total of 333 plants were used which were allocated nine each to the 37 treatments. Plants were placed in rows at vertical and horizontal distances of $1\frac{1}{2}$ ft from each other. When they were 6 to 8 in. high, a 2-oz application of ammonium sulphate was given to each plant. Prior to the time of flowering, each plant again received 2 oz of ammonium sulphate.

Solutions of the compounds were prepared for administration to the cauliflower plants in the following manner: NOXA[3] and IBA[3] were first dissolved

[2]Sutton and Sons, Ltd.
[3]British Drug Houses, Ltd.

in 95% alcohol, after which distilled water was added. The concentrations, in p.p.m., have been indicated in the previous section. The total amount of a compound used per plant depended upon the combination of concentration and frequency. For each combination of concentration and frequency, Table I gives the total amount of compound used, as a multiple of 10^{-8} g. As it precipitated when water was added, TIBA[4] could not be prepared in the same way as NOXA and IBA. TIBA was found to dissolve successfully in a pure olive oil base. The solutions were prepared so that 6 drops of TIBA (in an oil base) contained the same amount of the compound as 1 cc of NOXA or IBA (dissolved in water). The solutions were stored in bottles and kept in a refrigerator when not in use. Both NOXA and IBA were applied to the cauliflower plants using a spray atomizer, while TIBA was applied with a dropper. Nineteen days after transplantation, the compounds were given over a 12-day period.

TABLE I

Total amount of compound used for each combination of concentration and frequency (in 10^{-8} g)

Concentration (p.p.m.)	Frequency of application		
	2	3	6
	Total amount of compound		
0.01	2	3	6
0.2	40	60	120
4.0	800	1,200	2,400
80.0	16,000	24,000	48,000

The following measurements were taken on each of the 333 cauliflower plants: (*a*) maximum height in inches of the plants at the time of the first application of treatment (referred to subsequently as 'initial height'); (*b*) diameter of the head of the flower, in centimeters, every other day; (*c*) final weight of the cauliflower head, in ounces; and (*d*) qualitative observations were made to determine if the flower was insect-infected, if the growth was powdery, or if leaves appeared in the flower.

Results

Table II gives the mean initial height, mean diameter of the flowers every other day, and mean and standard deviation of final flower weight for the 37 treatments. The number of plants on which the diameter means are based is also given, which also indicates the number of plants which had flowered. In Fig. 1, mean diameter is plotted against time for the most effective treatment (in terms of mean final weight) for the three compounds and for the control group. Actual dates have been included in reporting the data to indicate the growing period of cauliflower under climatic conditions in India.

[4]L. Light and Co., Ltd.

TABLE II

Initial height, diameter of developing flowers, and final flower weight

Treatment No.	Compound	Concentration, p.p.m.	Frequency	Mean initial height, inches	Day/month								Weight, ounces	Final number of flowers
					26/11	28/11	30/11	2/12	4/12	6/12	8/12	10/12		
					Flower diameter in centimeters									
1	NOXA	0.01	2	17.22	M* 0	4.00	4.80	5.05	5.81	11.31	15.28	15.41	M 13.00	9
					n* 0	1	2	6	7	8	8	8	s 5.43	
2	NOXA	0.01	3	16.11	M 0	2.50	4.43	4.92	7.03	12.08	15.33	16.93	M 14.33	9
					n 0	3	3	6	6	6	7	8	s 5.67	
3	NOXA	0.01	6	15.00	M 4.00	8.50	7.90	7.53	15.30	14.23	14.65	13.76	M 13.06	9
					n 1	1	2	3	2	4	6	8	s 4.64	
4	NOXA	0.2	2	17.17	M 3.50	5.20	7.90	10.00	12.20	13.95	11.63	13.54	M 17.39	9
					n 1	1	1	1	1	2	6	7	s 4.83	
5	NOXA	0.2	3	15.72	M 0	0	2.00	3.43	4.87	8.73	10.71	14.24	M 11.94	9
					n 0	0	1	4	3	6	9	9	s 2.33	
6	NOXA	0.2	6	15.94	M 0	0	6.20	6.85	7.70	10.88	12.27	14.16	M 13.33	9
					n 0	0	1	2	2	4	7	8	s 8.68	
7	NOXA	4.0	2	13.39	M 0	0	0	2.50	5.28	8.03	10.43	14.15	M 14.50	9
					n 0	0	0	1	4	6	7	8	s 6.15	
8	NOXA	4.0	3	14.39	M 0	2.00	5.80	4.43	6.87	8.42	12.21	15.11	M 16.56	9
					n 0	1	1	3	3	6	7	8	s 7.73	
9	NOXA	4.0	6	15.17	M 0	0	0	5.60	4.83	7.83	9.06	11.68	M 14.61	9
					n 0	0	0	2	3	3	5	6	s 6.37	
10	NOXA	80.0	2	15.94	M 4.00	6.80	9.30	7.45	8.60	13.20	16.03	18.13	M 12.33	9
					n 1	1	1	2	2	3	4	4	s 3.98	
11	NOXA	80.0	3	15.00	M 0	0	0	3.97	6.13	11.12	12.71	15.83	M 12.67	9
					n 0	0	0	3	4	5	7	8	s 6.33	
12	NOXA	80.0	6	16.17	M 3.25	4.78	6.33	8.10	11.33	17.60	18.20	19.01	M 18.11	9
					n 2	5	6	6	6	6	6	7	s 6.58	
13	TIBA	0.01	2	15.67	M 0.30	2.50	6.16	6.38	8.15	10.84	13.49	15.76	M 12.50	9
					n 1	4	3	4	4	5	7	7	s 4.40	
14	TIBA	0.01	3	15.39	M 0	4.50	6.20	4.73	4.96	9.27	11.53	12.94	M 10.17	9
					n 0	1	1	4	5	7	8	9	s 4.75	
15	TIBA	0.01	6	15.11	M 0	2.00	3.00	3.33	4.25	8.20	12.38	14.26	M 11.00	9
					n 0	2	2	3	4	6	6	7	s 6.13	
16	TIBA	0.2	2	13.78	M 0	0	2.20	4.00	4.77	6.23	10.65	13.57	M 9.00	9
					n 0	0	1	1	3	6	6	6	s 5.08	
17	TIBA	0.2	3	13.44	M 0	1.93	2.92	4.15	6.14	9.54	13.31	17.20	M 11.01	9
					n 0	3	4	6	7	7	7	7	s 2.56	
18	TIBA	0.2	6	15.78	M 2.00	3.80	4.53	4.18	6.43	10.26	12.37	15.99	M 11.28	9
					n 1	3	4	4	6	8	9	8	s 7.22	
19	TIBA	4.0	2	14.93	M 2.00	2.02	3.12	3.67	5.09	9.80	13.01	16.30	M 9.94	9
					n 1	5	6	7	8	8	8	8	s 3.89	
20	TIBA	4.0	3	15.56	M 2.00	3.25	4.23	5.17	6.38	9.13	12.21	13.78	M 11.92	9
					n 1	2	3	3	5	7	8	9	s 5.21	
21	TIBA	4.0	6	15.39	M 0	1.47	2.53	2.92	4.38	7.45	11.25	15.47	M 9.56	9
					n 0	3	4	6	6	6	6	6	s 3.85	
22	TIBA	80.0	2	16.83	M 0	2.02	3.80	4.37	5.50	10.57	13.29	15.95	M 11.17	9
					n 0	5	4	6	6	6	7	8	s 4.69	
23	TIBA	80.0	3	16.89	M 2.25	3.13	4.23	4.67	5.88	10.06	13.04	16.31	M 10.50	9
					n 2	3	4	3	6	8	7	8	s 3.98	

24	TIBA	80.0	6	15.44	M	3.00	3.00	4.55	4.83	5.64	12.38	14.27	15.99	M 15.28	9
					n	1	3	4	6	7	8	9	9	*s* 5.57	
25	IBA	0.01	2	14.78	M	0	2.00	4.30	4.60	4.71	11.94	15.23	18.31	M 14.06	9
					n	0	1	1	3	7	7	7	7	*s* 2.59	
26	IBA	0.01	3	15.06	M	2.50	4.50	6.01	5.85	8.50	11.70	13.37	15.33	M 11.61	9
					n	1	1	1	4	3	4	6	7	*s* 3.02	
27	IBA	0.01	6	15.17	M	0	0	0	3.83	5.38	9.16	12.63	17.18	M 12.67	9
					n	0	0	0	3	5	5	6	6	*s* 5.44	
28	IBA	0.2	2	15.39	M	0	4.40	5.60	4.54	5.97	11.49	14.40	15.71	M 13.33	9
					n	0	1	2	5	7	7	7	8	*s* 4.58	
29	IBA	0.2	3	16.67	M	2.00	0	0	4.73	5.35	9.47	13.28	16.39	M 15.11	9
					n	1	0	0	3	6	7	8	9	*s* 5.24	
30	IBA	0.2	6	14.94	M	2.00	4.40	6.80	8.00	8.27	12.37	13.42	16.66	M 12.17	9
					n	1	1	1	1	3	3	5	5	*s* 6.29	
31	IBA	4.0	2	16.44	M	0	6.80	9.30	4.15	10.90	12.80	15.80	18.33	M 15.61	9
					n	0	1	1	2	2	5	6	6	*s* 4.07	
32	IBA	4.0	3	17.00	M	0	5.80	7.90	5.87	8.17	12.78	12.09	15.32	M 15.17	9
					n	0	1	1	3	3	4	8	9	*s* 5.61	
33	IBA	4.0	6	17.67	M	0	3.50	5.90	4.77	6.93	11.96	15.66	19.57	M 16.11	9
					n	0	1	1	6	6	7	7	7	*s* 6.22	
34	IBA	80.0	2	16.28	M	0	4.80	5.95	4.75	7.40	10.43	13.40	17.54	M 14.72	9
					n	0	1	2	4	4	7	7	7	*s* 5.25	
35	IBA	80.0	3	14.56	M	0	3.40	5.70	5.24	7.20	13.28	15.96	18.30	M 13.33	9
					n	0	3	3	5	5	6	7	8	*s* 5.00	
36	IBA	80.0	6	17.06	M	0	2.50	4.60	4.36	6.78	11.70	13.74	15.85	M 17.94	9
					n	0	1	1	5	5	6	9	8	*s* 5.18	
37	Control			16.39	M	3.00	4.85	6.50	6.65	7.80	12.50	11.76	12.46	M 12.39	9
					n	2	2	2	4	3	4	7	7	*s* 6.93	

*(M) Mean, (*n*) number of flowers, and (*s*) standard deviation.

TABLE III

Analysis of variance
Summary table for final cauliflower weight

Part A

Source of variation	d.f.	Sums of squares	Mean square	*F*
Columns	8	623.29	77.91	3.084**
Rows (unadjusted)	36	2298.83		
Treatments (adjusted)	36	1644.79	45.689	1.809**
Error	252	6365.42	25.26	
Total	332	10932.33		

Part B

Source of variation	d.f.	Sums of squares	Mean square	*F*
Columns	8	623.29		
Rows (adjusted)	36	2208.51	61.348	2.429**
Treatments (unadjusted)	36	1735.11		
Error	252	6365.42		
Total		10932.33		

NOTE: Critical difference: $2 \times 3 \times 1.96 \times \sqrt{37 \times 25.26} = 357.1688$ for K $(Qj - Qj')$
**$P < .01$

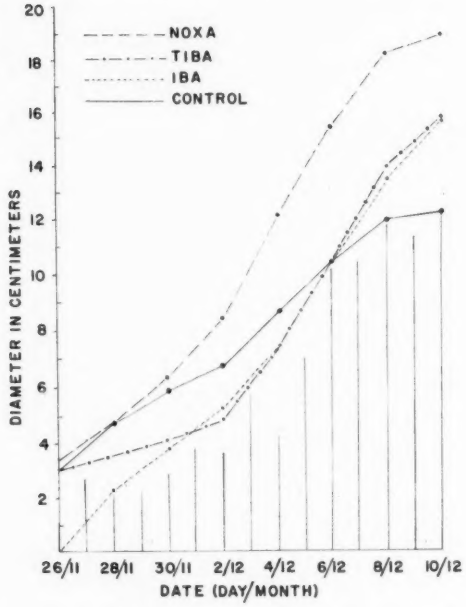

FIG. 1. Comparative increase in mean cauliflower diameter under NOXA, TIBA, and IBA treatments, at 80.0 p.p.m. applied six times, and for the untreated control group.

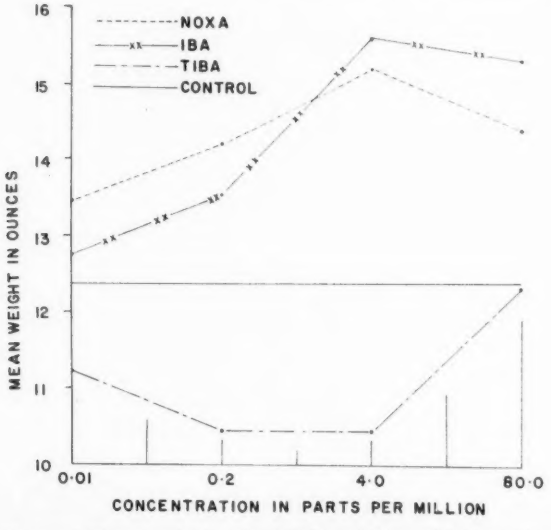

FIG. 2. Mean cauliflower weight under NOXA, TIBA, and IBA treatments at four concentration levels, in p.p.m., along with control group mean.

Before testing the effectiveness of the experimental treatments, it was necessary to determine whether the plants in the different groups were comparable. For this purpose, measurements of initial height were taken. An analysis of variance of these measurements showed that the groups did not differ significantly prior to the experimental treatments. Statistical significance of the results was examined by an analysis of variance on the final flower weight. As a Youden square design was employed, it was necessary to adjust the treatment yields before testing for significance. The analysis of variance summary table given in Table III tested the differences after making the required adjustments of the treatment yields, and showed that the treatments differed significantly (Part A of Table III). The analysis also showed that the design controlled effects due to rows (which was indicated by a significant F in Part B of Table III) and columns (by a significant F in Part A of Table III). To determine which treatments differed significantly from the control, the critical difference required for significance was computed and included in Table III. (The critical difference was computed for the adjusted yields which are not reported due to space restrictions.) For the following four treatments, final weight differed significantly ($P<.05$) from the control: (*a*) NOXA at 0.2 p.p.m. applied twice; (*b*) NOXA at 4.0 p.p.m. applied thrice; (*c*) NOXA at 80.0 p.p.m. applied six times; and (*d*) IBA at 80.0 p.p.m. applied six times. The mean flower weight for the control group and for the three compounds at four concentration levels (obtained for each concentration by averaging its three frequency groups) is presented graphically in Fig. 2. A frequency polygon (not presented here) drawn for all 333 plants showed the distribution of flower weight to be unimodal and symmetric.

Qualitative observations indicated that a high proportion of plants treated with TIBA had leaves present in their flowers. As TIBA was dissolved in olive oil, mention may be made of previous experiments in this laboratory, in which it was observed that olive oil alone had an effect on the shape and color of cauliflower leaves, making them wavier and darker.

Discussion

One aspect of cauliflower growth which may be of considerable importance commercially is the flower weight. Statistical analysis of the results in this study shows that for four treatments with synthetic growth-promoting substances, final weight was significantly greater than that of the control group. The mean weights for these four treatments, which used NOXA and IBA, range from 16.56 to 18.11 oz, as compared with 12.39 oz for the control group. When all frequencies are combined for a given concentration (see Fig. 2), 4.0 p.p.m. stands out as the most effective concentration level. Figure 2 also shows that for TIBA, none of the concentration levels employed raised the mean weight above that of the control. Further experiments using NOXA and IBA are particularly suggested by these data.

In order to obtain some information concerning the pattern of flower growth, successive measurements of flower diameter were taken. In the con-

trol group, there was a rapid increase in diameter during the first 12 days of measurement, after which diameter did not increase. For the treated plants, there was also a rapid increase in diameter; however, the levelling off was not observed (see Fig. 1). The highest terminal values of diameter were obtained for NOXA, second highest for IBA, and lowest for TIBA. These results suggest that synthetic growth-promoting substances may prolong the period of flower expansion, as noted in grapes, oranges, apricots, and other fruits (3).

From a commercial point of view, it may be noted that the increase in weight of the successfully treated flowers over the control ranged, on the average, from 34 to 46%. These results suggest that synthetic growth-promoting substances, which have already demonstrated their value for other crops, may also be profitable for commercial cauliflower cultivation.

Acknowledgments

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ON THE GENERA MORMOLYCA FENZL AND CYRTOGLOTTIS SCHLTR.[1]

LESLIE A. GARAY[2] AND MICHAEL WIRTH[3]

Abstract

The generic limits of *Mormolyca* Fenzl are extended to include *Cyrtoglottis* Schltr., on the basis of intermediate species. A previously misunderstood species, *Maxillaria galeata* Scheidw., is included in the genus *Mormolyca*, and the relationship of this genus to the rest of the *Maxillaria*-alliance is discussed. One new species, *Mormolyca polyphylla*, is described, and a synopsis of the genus is presented.

Introduction

In 1956, the Swedish Academy of Science, in co-operation with the State Museum of Natural History, undertook an expedition to Ecuador to explore the Napo-Pastaza Province, which is known to be rich in botanical endemics. The Orchid Herbarium of Oakes Ames at Harvard University was recently requested to study and identify all orchidaceous plants collected by this expedition. The collection includes a number of important species, which, when the information they provide is properly evaluated, will contribute much toward a clearer understanding of relationships between certain genera.

In this paper we intend to discuss one of the taxonomic problems which became apparent during our study of the material from this expedition; i.e., the circumscription, status, and relationship of the genera *Mormolyca* and *Cyrtoglottis*.

One of these collections, *Asplund No. 19538*, appeared to be closely related to the genus *Cyrtoglottis* Schltr. In examining the floral details, however, we discovered that the material at hand has so many characteristics of both *Cyrtoglottis* and *Mormolyca* that a consideration of the validity of maintaining these genera was prompted.

Discussion

The genus *Mormolyca* was established by Fenzl (1) with one species, *M. lineolata*, from Mexico. This taxon, however, proved to be identical with the earlier-described *Trigonidium ringens* Lindl. (4) and thus became correctly designated as *M. ringens* (Lindl.) Schltr.

Schlechter proposed the genus *Cyrtoglottis* in 1920 (6), based on a collection from Colombia, indicating its close relationship to *Mormolyca*, but separating the two taxa primarily on the basis of the geographic disjunction as well as on the filiform petals and the acuminate sepals of *Cyrtoglottis*.

[1]Manuscript received February 2, 1959.
Contribution from Botanical Museum, Harvard University, Cambridge, Massachusetts, U.S.A.

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The geographic separation of the two genera was shown to be invalid by the discovery of *Mormolyca peruviana* C. Schweinf. (7), which extended the range of the genus from Central America to South America, thus rendering the two genera spacially contiguous.

On the basis of floral morphology, the two taxa might be maintained, were it not for the new species described herein: *Mormolyca polyphylla*, from Ecuador. Although vegetatively it is very distinct, the floral details are intermediate between those of *Mormolyca* and *Cyrtoglottis*. *Mormolyca polyphylla* has the typical lip and column of *Cyrtoglottis*; the petals are narrower than the sepals, but they are not filiform; furthermore, the sepals are acute, rather than long-acuminate, thus closely approaching the features of *Mormolyca*. Therefore, the new species, on the basis of floral characters (the acute sepals and non-filiform petals) is assignable to *Mormolyca*, whereas in the structure of the lip and column, it is referable to *Cyrtoglottis*. Hence it seems necessary to consider *Cyrtoglottis* as a synonym of *Mormolyca*.

Another addition to the genus *Mormolyca* appeared to be necessary when we reviewed the species of the whole *Maxillaria*-alliance. The original description of *Maxillaria galeata* by Scheidweiler (5), as well as the illustration of this species by Kraenzlin in Xenia Orchidacea (3), do not disclose the presence of a column-foot. Furthermore, the callus of the lip is rather more like that of *Mormolyca* than of *Maxillaria*, and the pollinia show the same long stipe and semi-lunate gland which are seen in *Mormolyca polyphylla*. In his treatment of *Maxillaria galeata* in Flora Brasilica, Hoehne (2) has included *Maxillaria atrata* Rchb. f. in synonymy, a step which is definitely incorrect, since *M. atrata* possesses a small but definite column-foot and is now regarded as a synonym of the Central American *Maxillaria cucullata* Lindl.

The absence of a column-foot in *Maxillaria galeata* renders its present position untenable. Consequently, it is necessary to transfer it to the genus *Mormolyca*. This raises the question of the relationship and status of *Mormolyca* to other members of the Maxillarieae which are characterized by a simple, footless column.

Six of the genera in the *Maxillaria*-alliance have been characterized by the absence of a column-foot. Two of these, *Chrysocycnis* Rchb. f. and *Trigonidium* Lindl., are incorrectly described and placed, inasmuch as they possess a small but definite column-foot and are, therefore, dubiously distinct from *Maxillaria* Ruíz & Pav. The genera *Cyrtidium* Schltr. and *Pityphyllum* Schltr. appear to be amply distinct, the former because of its pleuranthous inflorescence, the latter because of the peculiar manner of leaf-bearing. The case of *Mormolyca* Fenzl and *Cyrtoglottis* Schltr. has just been discussed.

The genus *Mormolyca* is closely related to *Trigonidium* and they may be differentiated from each other by the presence of a column-foot and prominent sepals which are connivent at the base into a tube in *Trigonidium*. However, in considering these two genera, a number of intermediates may be found, particularly with reference to *Mormolyca polyphylla* and *Trigonidium grande* Garay. Both of these species represent a somewhat aberrant type vegetatively, having in common a polyphyllous pseudobulb.

The delimitation of genera, as at present held, is far from being satisfactory. *Mormolyca* and *Trigonidium* ultimately may prove to be inseparable from *Maxillaria*, especially when sufficient research is carried out with regard to cytotaxonomy of the whole subtribe Maxillarieae.

Mormolyca Fenzl

MORMOLYCA Fenzl in Denkschr. Acad. Wien, Math. Nat. 1: 253, 1850; Bentham in Benth. & Hooker, Gen. Pl. 3: 554, 1883; Pfitzer in Engler & Prantl, Die Natürliche Pflanzenfam. 2, pt. 6: 186, 1888; Schlechter, Die Orchideen 436, 1914; ed. 2, 431, 1927; Lemée, Dict. 4: 568, 1932; Ames in Field Mus. Nat. Hist. Bot. 18: 261, 1937; L. O. Williams in Ceiba 2: 266, 1951; ibidem 5: 210, 1956; Ames & Correll in Fieldiana 26: 590, 1953.

Syn.: *Cyrtoglottis* Schltr. in Fedde Rep. Beih. 7: 181, 1920.

Mormolyce Fenzl ex Bentham in Benth. & Hooker Gen. Pl. 3: 554, 1883, sphalm.

Mormolyze Fenzl ex Schltr., Die Orchideen 436, 1914, sphalm.

Sepala subaequalia, ringentia; sepalum posticum a sepalis lateralibus omnino liberum, concavum; sepala lateralia a basi patentia, decurva. Petala sepalis minora. Labellum a basi columnae porrectum, simplex vel trilobum; lobi laterales erecti vel recurvi; lobus intermedius porrectus vel recurvus; discus sive per totam longitudinem incrassatus sive in medio callo ornatus. Columna plus minusve arcuata, clavata, exalata, apoda; clinandrium vix rite evolutum; rostellum valde abbreviatum, emarginatum; anthera terminalis, opercularis, incumbens; pollinia 4, cerea, ovoidea, per paria sibimet incumbentia vel connata, aequalia vel interdum inaequalia, glandulae squamiformi hippocrepicae cum stipite affixa.—Herba epiphytica; rhizomate repenti; pseudobulbis aggregatis vel plus minusve remotis, apice 1–4-foliatis; folio carnoso vel coriaceo, basi conduplicato; inflorescentia singula vel plurima, juxta basin pseudobulborum nata, uniflora.

Typus: TRIGONIDIUM RINGENS Lindl.

CLAVIS SPECIERUM

1	Pseudobulbi 3-vel 4-foliati	*M. polyphylla*
1′	Pseudobulbi 1-vel 2-foliati	2
2	Petala filiformia, quam sepala multa angustiora	3
2′	Petala sepalaque plus minusve similia	4
3	Labellum prominenter 3-lobum	*M. Schweinfurthiana*
3′	Labellum integrum, obovatum, apiculatum	*M. gracilipes*
4	Lobi laterales labelli valde producti, elongati, oblique falcato-lanceolati	*M. peruviana*
4′	Lobi laterales labelli abbreviati, ovato-triangulares	5
5	Pseudobulbi bifoliati; callus labelli bisulcatus	*M. galeata*
5′	Pseudobulbi unifoliati; callus labelli integer seu obscure lobatus, numquam sulcatus	*M. ringens*

DESCRIPTIONES

Mormolyca galeata (Scheidw.) Garay & Wirth, comb. nov.

Syn: *Maxillaria galeata* Scheidw. in Otto & Dietr. Allg. Gartenzeit. 10: 309, 1842; Reichenbach f. in Walp. Ann. Bot. Syst. 6: 526, 1863; Kränzlin in Xenia Orchid. 3: 130, t. 274, fig. 3, 1894; Cogniaux in Mart. Fl.

Bras. 3, pt. 6; 58, 1904; Hoehne, Fl. Brasil. 12, pt. 7: 339, t. 176, 1953, excl. syn. Fig. 1.

Epiphytica, usque ad 30 cm alta; rhizomate satis abbreviato; pseudobulbis aggregatis, anguste oblongo-cylindraceis leviter compressis, apice diphyllis, 3–4 cm altis; foliis anguste ligulatis, apice acutis, basi satis conduplicatis, 15–25 cm longis, 1–2 cm latis; pedunculis plurimis, erectis, gracilibus, vaginis remotis, subventricosis obsessis, 6–8 cm longis; floribus ringentibus, purpureo-punctatis; sepalo postico oblongo-ovato vel lanceolato-ovato, apice acuto, satis concavo, 2–2.5 cm longo, 6–7 mm lato; sepalis lateralibus obliquis, sepalo postico simillimis et cum eo aequilongis; petalis lanceolatis, paulo obliquis, apice acutis vel breviter acuminatis, 2.2–2.3 cm longis, 5–6 mm latis; labello carnoso, 3-lobo; lobis lateralibus ovato-triangularibus, apice acutiusculis; lobo intermedio oblongo-linguiformi, apice acuto; disco basi calloso, callo brevi, bisulcato, apice subtruncato; toto labello 2 cm longo, inter lobos laterales 7–8 mm lato; columna brevi, crassa; anthera galeata (unde nomen).

Brazil: Without a definite locality. Coll.: *Scheidweiler s.n.* (Type): São Paulo: *Comm. Geigr. e Geol. S. Paulo No. 1864* (SP); ex cult. *Wendland s.n.* (W).

FIG. 1. *Mormolyca galeata* (Scheidw.) Garay & Wirth. Side and front view of the flower, and lateral view of the lip. Redrawn from Kränzlin, Xenia Orchidacea 3: t. 274, Fig. 3, 1894.

Mormolyca gracilipes (Schltr.) Garay & Wirth, comb. nov.

Syn.: *Cyrtoglottis gracilipes* Schltr. in Fedde Rep. Beih. 7: 182, 1920; Mansf. in Fedde Rep, Beih. 57: t. 65, nr. 250, 1929; Schweinf. in Bot. Mus. Leafl. Harvard Univ. 12: 185, 1946. Fig. 2.

Epiphytica, caespitosa, usque ad 27 cm alta; radicibus crassiusculis, leviter flexuosis, glabris; pseudobulbis aggregatis, anguste oblongo-ovatis, vel oblongo-ellipticis, lateraliter compressis, apice unifoliatis, 1.5–4 cm altis; folio elliptico vel ovato-elliptico, apice acuto, basi plus minusve conduplicato, satis coriaceo, 5.5–11.5 cm longo, 1.3–2.6 cm lato; pedunculo erecto, satis gracili, unifloro, 8.5–27 cm longo; floribus ringentibus; sepalo postico valde cucullato, late ovato-lanceolato, apicem versus attenuato, 1.6–3.2 cm longo, 0.9–1.1 cm lato; sepalis lateralibus oblique ovato-lanceolatis, apicem versus longe attenuatis, 1.8–3.4 cm longis, 0.9–1 cm latis; petalis filiformibus, apice acutis, 0.9–1.6 cm longis, 1–1.3 mm latis; labello satis carnoso, rhombeo-obovato, antice rotundato et in medio breviter apiculato, lateraliter recurvo, glabro vel interdum margine obscure ciliato, disco in medio longitudinaliter incrassato, supra basin callo vix evoluto ornato, 0.8–1.1 cm longo, 6–8 mm lato; columna arcuato-clavata, 7–9 mm longa, facie glabra vel subtiliter ciliolata.

Colombia: Cauca. Altitude about 1200 meters. Coll.: *M. Madero s.n.* (Type); ibidem *F. C. Lehmann No. 813* (AMES) Neotype.

Ecuador: Prov. Napo-Pastaza, Mera. Coll.: *E. Asplund No. 19535* (S); Esmeraldas. Coll.: *F. C. Lehmann No. 231* (G).

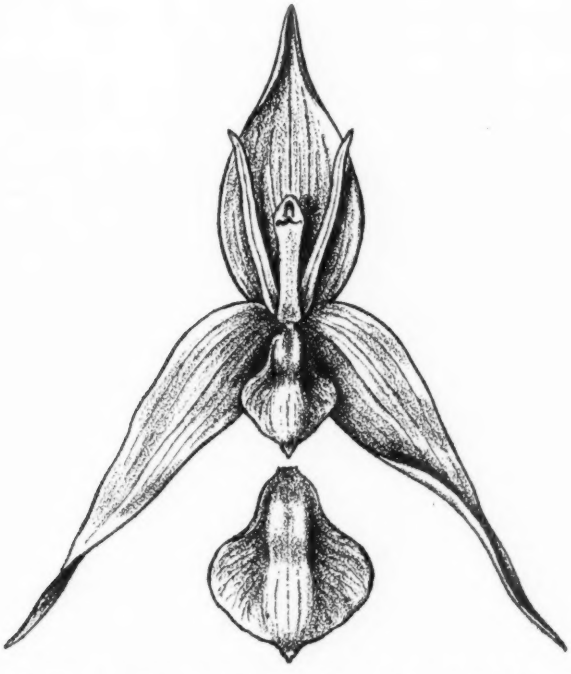

FIG. 2. *Mormolyca gracilipes* (Schltr.) Garay & Wirth. Front view of the flower, ×2. Expanded lip, ×3.

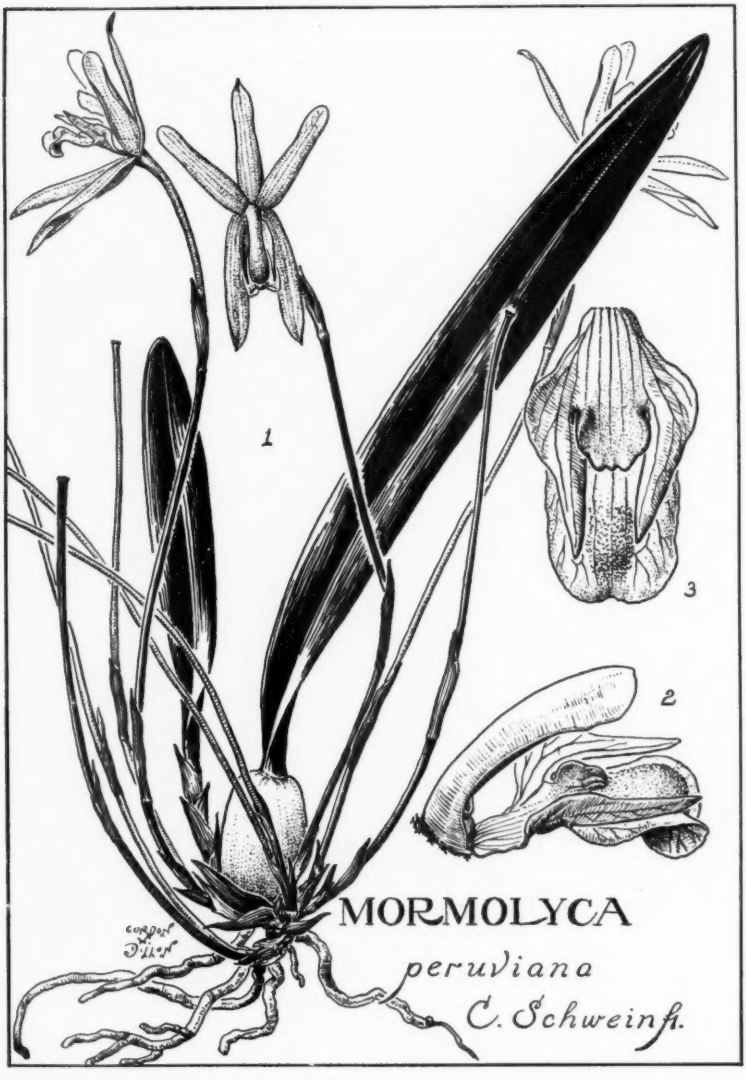

FIG. 3. *Mormolyca peruviana* C. Schweinf. 1. Habit of plant, natural size. 2. Lip and column from side, ×3. 3. Lip from above, ×3.

Peru: Dept. Junín. Chanchamayo Valley. Coll.: *C. Schunke No. 1119* (F), *No. 1734* (F); San Ramón. Coll.: *Killip & Smith No. 24883* (US); Prov. Cuzco. Convención. Coll.: *C. Vargas No. 3257a* (AMES); Quispicanchis. Coll.: *C. Vargas No. 3125* (AMES).

Mormolyca peruviana C. Schweinf. in Amer. Orch. Soc. Bull. 13: 196, t., 1944. Fig. 3.

Epiphytica, caespitosa, usque ad 16 cm alta; radicibus fasciculatis, leviter flexuosis, glabris; rhizomate valde abbreviato; pseudobulbis subquadrato-ovoideis, complanatis, apice unifoliatis, ca. 2 cm altis; folio lineari-oblongo, apice acuto vel obtusiusculo, basi sessili, satis coriaceo, nervo mediano subtus satis prominenti, 14 cm longo, 1.2 cm lato; pedunculis plurimis, erectis vel paulo arcuatis, gracilibus, in medio univaginatis, apice unifloris; flore ringenti, margine sepalorum petalorumque valde revoluto; sepalo postico lanceolato-oblongo, apice acuto et breviter mucronato, ca. 2 cm longo, 5 mm lato; sepalis lateralibus oblique oblongo-lanceolatis, apice acutis et breviter mucronatis, 2.2 cm longis, 6 mm latis; petalis lineari-oblongis, apice acutis vel obtusis, ca. 1.9 cm longis, 4.5 mm latis; labello erecto, columnae parallelo, e basi cuneata 3-lobo; lobis lateralibus oblique lanceolato-triangularibus, apice acutis vel acuminatis; lobo intermedio subquadrato, apice rotundato-subtruncato, interdum breviter retuso; disco supra medium callo late ovato, antice tridentato ornato; toto labello 1.7 cm longo, 7 mm lato; columna arcuata, ca. 1.3 cm longa.

Peru: Dept. Junín, Chanchamayo Valley. Altitude 1800 meters. Coll.: *C. Schunke No. 564.* Type. (F, AMES).

Mormolyca polyphylla Garay & Wirth, sp. nov. Fig. 4.

Epiphytica, erecta, usque ad 60 cm alta; rhizomate valde abbreviato; radicibus crassiusculis, leviter flexuosis, glabris; pseudobulbis oblongo-cylindraceis, lateraliter paulo compressis, apice 3–4-phyllis, 12–23 cm longis, 1.5–2 cm crassis; foliis oblongo-ellipticis, utrinque attenuatis, apice acutis seu abrupte acuminatis, basi in petiolum brevem conduplicatis, 20–40 cm longis, 4–6 cm latis; pedunculis succedaneis, juxta basin pseudobulborum natis, satis gracilibus, erectis vel paulo arcuatis, vaginis remotis arcte amplectentibus obsessis, apice unifloris, usque ad 20 cm altis; flore satis conspicuo, ochraceo, brunneo-striato; sepalo postico ovato-lanceolato, valde cucullato, dorsaliter obscure carinato, apice breviter mucronato, 2.5–3.3 cm longo, 7–8 mm lato; sepalis lateralibus oblongo-falcatis, apicem versus sensim attenuatis, 2.6–3.2 cm longis, 7–8 mm latis; petalis ovato-lanceolatis, apice acuminatis, 2–2.6 cm longis, 5–7 mm latis; labello carnosissimo, satis complicato, vi expanso, subtrilobo; lobo intermedio et lobis lateralibus marginem versus densissime hirsutis; lobo intermedio carnosissimo, antice recurvo, obtuso seu rotundato, lobis lateralibus tenuibus, reflexis, disco per totam longitudinem incrassato, in medio callo bipartito ornato; toto labello 8–10 mm longo, inter lobos laterales 4–5 mm lato; columna arcuata, crassa, apice clavata, facie subtiliter villosa; ovario cylindrico, prominenter 6-alato, cum pedicello 2–2.5 cm longo.

Ecuador: Prov. Napo-Pastaza, Mera, epiphyte in *Crescentia cujete* tree. Altitude about 1100 meters. March 2, 1956. Coll.: *Erik Asplund No. 19538.* Type! (S); Oriente. Coll.: *Strobel s.n.* Cultivated at the Montreal Botanical Garden. (AMES).

The above description was prepared from the type specimen and augmented by the study of living material.

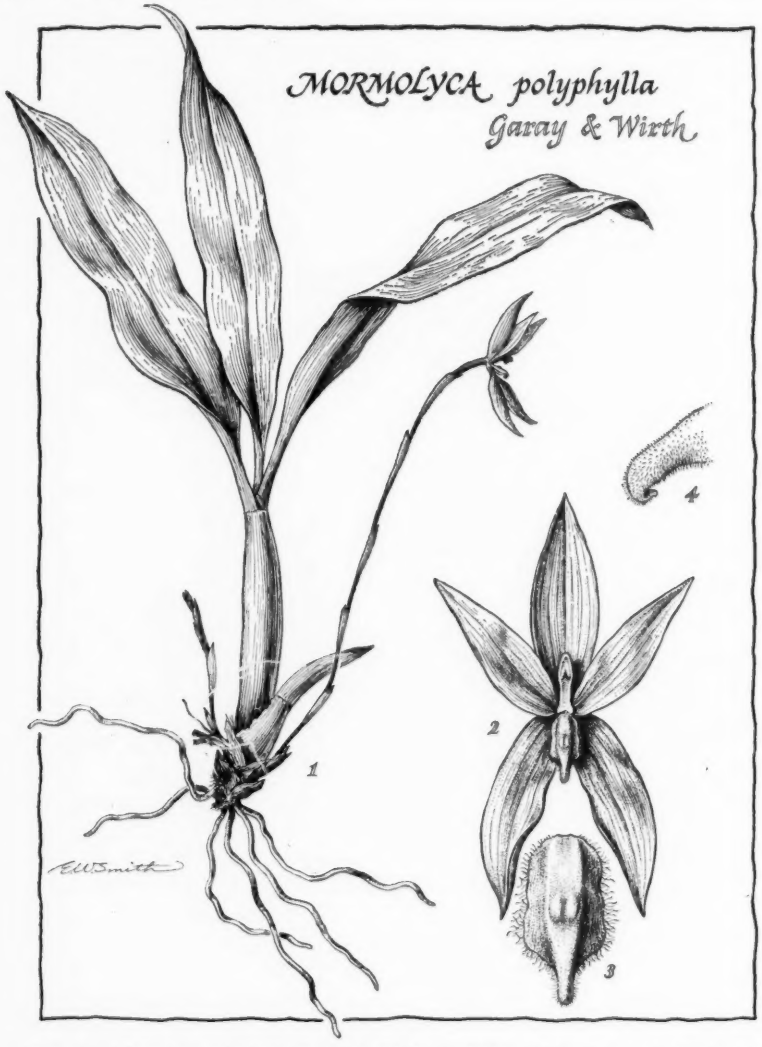

FIG. 4. *Mormolyca polyphylla* Garay & Wirth. 1. Habit of plant, ×½. 2. Expanded flower, ×1. 3. Lip, ×2½. 4. Apex of lip in natural position, much enlarged.

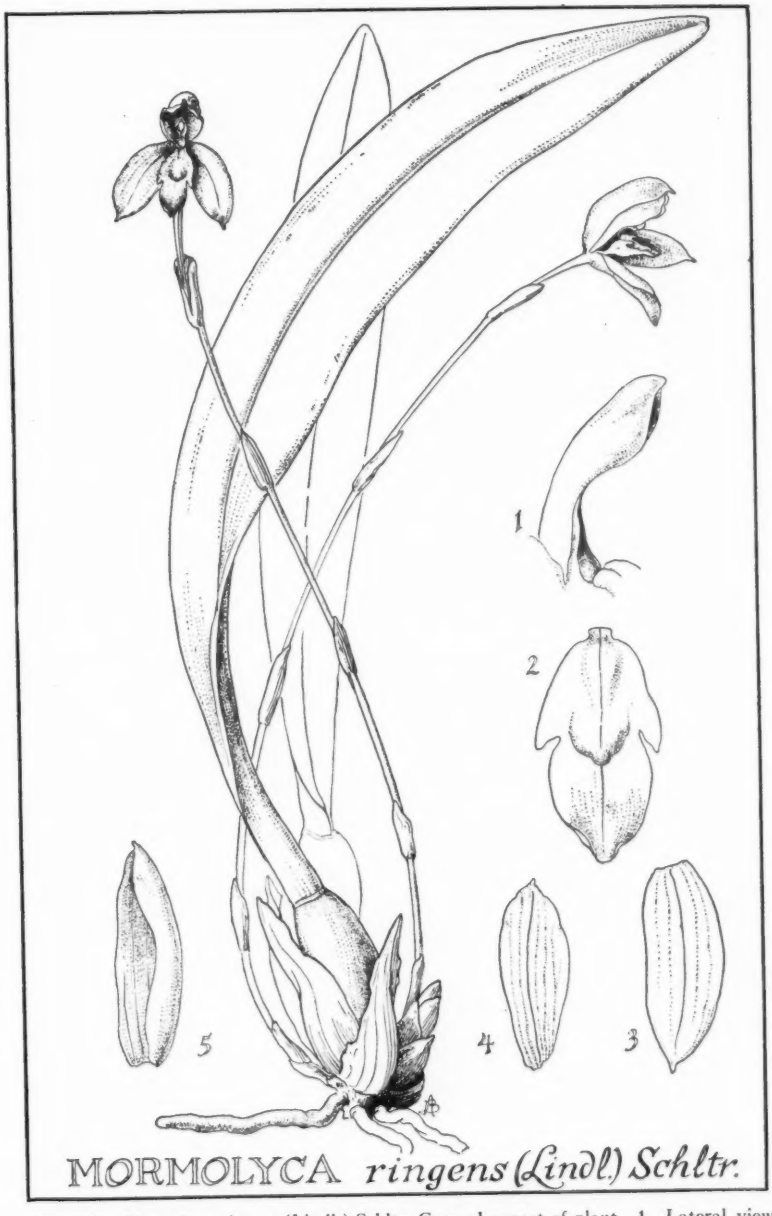

FIG. 5. *Mormolyca ringens* (Lindl.) Schltr. General aspect of plant. 1. Lateral view of column, ×2. 2. Lip from above, ×3½. 3. Lateral sepal, ×1½. 4. Petal, ×2. 5. Dorsal sepal, ×1½.

Mormolyca ringens (Lindl.) Schltr., Die Orchideen 436, 1914; ed. 2, 431, 1927; Ames in Field Mus. Nat. Hist. Bot. 18: 261, 1937; L. O. Williams in Ceiba 2: 266, 1951; ibidem 5: 210, 1956; Ames & Correll in Fieldiana 26: 590, 1953.

Syn.: *Trigonidium ringens* Lindl. in Bot. Reg. 26: Misc. p. 57, 1840; in Paxt. Fl. Gard. 3: 61, 1852–53; Reichenbach f. in Walp. Ann. Bot. Syst. 3: 541, 1852; ibidem 6: 504, 1863.

Mormolyca lineolata Fenzl in Denkschr. Acad. Wien, Math. Nat. 1: 253, t. 29, 1850. Fig. 5.

Epiphytica, caespitosa, usque ad 25 cm alta; rhizomate satis abbreviato; radicibus crassiusculis, glabris; pseudobulbis approximatis seu inter se usque ad 2 cm distantibus, globosis vel ovoideis, lateraliter compressis, monophyllis, 1.3–4 cm altis; foliis oblongo-ligulatis, interdum ovato-lanceolatis, apice acutis, basi complicatis, nervo mediano subtus valde prominenti, 6.3–35 cm longis, 1.3–3.5 cm latis; pedunculis plurimis, juxta basin pseudobulborum natis, erectis vel paulo arcuatis, vaginis remotis infundibuliformibus obsessis, unifloris, 6–37 cm longis; floribus ringentibus, virescentibus purpureo-suffusis interdum brunneo-striatis; sepalo postico valde cucullato, oblongo-elliptico, apice rotundato vel acuto, dorsaliter apicem versus carinato-mucronato, cum petalis galeam formanti 1.4–1.9 cm longo, 6–8 mm lato; sepalis lateralibus obliquis, oblongo-falcatis seu ellipticis, apice acutis vel obtusis, breviter mucronatis, 1–1.7 cm longis, 4.5–7 mm latis; petalis ellipticis vel oblique obovatis, apice acutis vel obtusis, minutissime vel brevissime mucronatis, 1–1.5 cm longis, 4–6 mm latis; labello 3-lobo; lobis lateralibus triangularibus vel ovato-triangularibus, apice acutis vel obtusiusculis, lobo intermedio semiorbiculari vel oblongo-elliptico, apice acuto vel obtuso, interdum breviter emarginato; disco in medio callo V-formi integro vel 3-lobato ornato; toto labello glabro vel marginem versus minutissime papilloso-ciliolato, 1 cm longo, inter lobis laterales 4–6 mm lato; columna clavata, leviter arcuata, basim versus paululo dilatata sed numquam pedem formantia, 0.8–1.3 cm alta; ovario pedicellato 2–4 cm longo.

Mexico: Without locality. Coll.: *Hartweg s.n.* (Type of *Trigonidium ringens*) (K); near Mirador. Coll.: *Heller s.n.* Cult. in Hort. Huegel (Type of *Mormolyca lineolata*) (W); Barranca de Tioselo. Coll.: *Schiede s.n.* (K.W.); Prov. Chiapas: Locunusco Mts. Coll.; *O. Nagel No. 4331* (AMES); East of Ocosingo. Coll.: *R. L. Dressler No. 1768* (AMES); Santa Catarina. Coll.: *R. Oberg No. 199* (AMES); Prov. Veracruz: Barranca de Zacuapan. Coll.: *Purpus No. 2144* (AMES); ibidem. Coll.: *O. Nagel No. 3774* (AMES); West of Tezonapa. Coll.: *O. Nagel No. 4141* (AMES), *No. 3595* (AMES); Near Paso del Toro. Coll.: *O. Nagel No. 4696* (AMES); Near Mwatitlan. Coll.: *O. Nagel No. 3650* (AMES); Laguna Encantada. Coll.: *Dressler & Jones No. 80* (AMES).

Guatemala: Alta Verapaz. Cubilguitz. Coll.: *v. Tuerckheim No. 8291* (AMES); Escuintla: Río Guacalate. Coll.: *P. C. Standley No. 60155* (AMES); ibidem. Coll.: *Hunnewell No. 14674* (F); Izabal. Coll.: *Lewis No. 79* (AMES); ibidem. Coll.: *Harris No. 79* (AMES); Jocolo, Lake Izabal. Coll.: *Johnson*

No. 1174 (AMES); Jalapa: Chahuite. Coll.: *P. C. Standley No. 77492* (F); Secetepéquez: Barranca Hondo. Coll.: *P. C. Standley No. 65037* (F).

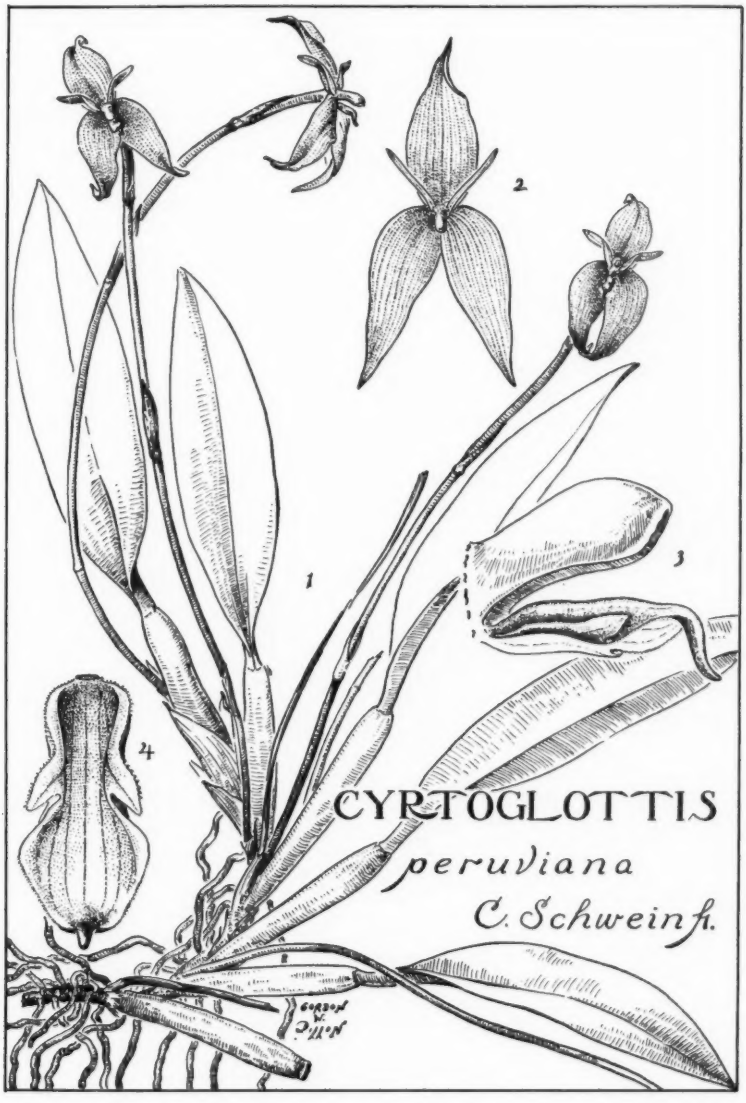

FIG. 6. *Mormolyca Schweinfurthiana* Garay & Wirth (described originally as *Cyrtoglottis peruviana* C. Schweinf.). 1. Habit of plant, ×2/3. 2. Flower with lip removed, natural size. 3. Column and lip, side view, ×4. 4. Lip, under surface, ×5.

British Honduras: El Cayo District. Coll.: *Gentle No. 2577* (AMES); Stann Creek Railway, 11 mile. Coll.: *Schipp No. 534* (AMES), *No. 535* (AMES).

Honduras: Cortes: Río Lindo. Coll.: *Williams & Molina No. 11382* (AMES) Santa Cruz de Yojoa. Coll.: *Edwards No. 533* (AMES), *No. 613* (AMES NY); Camayagua: Pito Solo, Lake Yojoa. Coll.: *Edwards No. 231* (AMES); Río Frio. Coll.: *Edwards No. 450* (AMES); Payatown, Plantain River. Coll.: *Spinden s. n.* (AMES); Atlantida: Lancetilla Valley. Coll.: *P. C. Standley No. 55427* (AMES), *No. 55251* (AMES); Guaymas, Coll.: *Ames 11.131* (AMES); Yora: Vicinity of Progresso. Col.: *Ames 11.119* (AMES).

Costa Rica: Guanacaste: Tilaran. Coll.: *Standley & Valerio No. 46574* (AMES), *No. 46108* (AMES), *No. 44539* (AMES).

Mormolyca Schweinfurthiana Garay & Wirth, nom. nov.

Syn.: *Cyrtoglottis peruviana* C. Schweinf. in Amer. Orch. Soc. Bull. 14: 152, t., 1945. Fig. 6.

Epiphytica, usque ad 20 cm alta; rhizomate prorepenti; radicibus filiformibus, flexuosis, glabris; pseudobulbis plus minusve remotis, anguste oblongo-ellipticis vel anguste cylindraceis, lateraliter compressis, apice unifoliatis, 2.2–6 cm altis; folio coriaceo, elliptico vel oblongo-elliptico, apice acuto, basi conduplicato, 6.2–9.6 cm longo, 1.5–2.8 cm lato; pedunculo succedaneo, gracili, erecto vel paulo arcuato, vaginis remotis obsesso, unifloro, 12–20 cm longo; flore ringenti; sepalo postico lanceolato-ovato, apicem versus attenuato, apice ipso acuto vel acuminato, 2.1–2.6 cm longo, 1–1.2 cm lato; sepalis lateralibus oblique ovato-lanceolatis, apice acuminatis, 2.2–2.8 cm longis, 1–1.1 cm latis; petalis lineari-filiformibus, paululo falcatis, 1.2–1.3 cm longis, 1–1.5 mm latis; labello convexo, 3-lobo, in ambitu obovato; lobis lateralibus minutis, triangularibus, acutis, lobo intermedio magno, ovato vel ovato-lanceolato, apice acuto et minutissime apiculato, apiculo reflexo; disco per totam longitudinem incrassato, margine lateraliter reflexo et minutissime ciliolato; toto labello 9–9.6 mm longo, inter lobos laterales (vi expanso) 5–6 mm lato; columna crassa, clavata, 7–9 mm longa.

Peru: Ccarrapa, between Huanta and Río Apurimac. Altitude about 1500 meters. Coll.: *Killip & Smith No. 23218*. Type. (AMES); Ayacucho, Aina, between Huanta and Río Apurimac. Altitude at 750–1000 meters. Coll.; *Killip & Smith No. 22595* (US); Dept. Junín; Tarma. Coll. *Woytkowski No. 35439* (AMES).

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NOTES

A NON-DESTRUCTIVE METHOD OF ESTIMATING MOISTURE IN WOOD[1]

D. E. ETHERIDGE[2]

Most methods of estimating moisture in biological material, such as wood, involve oven-drying, but the process is destructive and excludes the possibility of periodically evaluating changes occurring in the moisture content of a particular ecological niche. The introduction of hygrometric methods for estimating moisture by measuring the humidity of the air in equilibrium with the material has proved effective for wood but only to the limit of saturation of the fibers, that is 27% of the ovendry weight, which corresponds to a relative humidity of 100%. Recently, in an attempt to evaluate the influence of branch moisture content on the infection process in living aspen, a method was developed which appears to overcome these difficulties.

The method is based on the principle that a hygroscopic substance, such as wood, will eventually acquire equilibrium with any hygroscopic material in contact with it. If the material to be measured is wood, an estimate of its moisture content can be obtained by measuring the water in a wood probe with which it has reached equilibrium.

Tests are carried out with cylindrical probes (1 to $1\frac{1}{2}$ in. in length), prepared from air-dry, $\frac{1}{4}$-in. birch dowelling, which are inserted in small holes bored in the wood to be measured. After equilibrium has been attained, the probes are removed, weighed, and the amount of water present is assumed to be equal to that in the sample. Laboratory trials have indicated that equilibrium between the probes and samples of wood occurs after a period of 8 days for moisture contents in the range 30 to 80% of the ovendry weight, and after 24 hours for moisture contents in the range 6 to 30%. In practice, the probes are left in the wood for a minimum of 8 days before removing them for measurement. The holes are bored in the wood by means of a hand drill equipped with a bit 1/32 in. larger than the diameter of the wood probe. The holes should be deep enough to accommodate the full length of the moisture probe. Generally, the probes can be removed with a dissecting needle, but forceps of the haemostat type are useful if excessive swelling has occurred. Best results are obtained when the end of the probe is sealed with grafting wax, but care must be exercised to prevent small particles of wax from adhering to the wood. A small piece of cellulose tape fixed to the end of the probe has been found satisfactory for this purpose.

For field trials it was found convenient to prepare a supply of variously sized wood probes in the laboratory. These were first weighed in equilibrium with the air, and their ovendry weights calculated on the basis of a

[1]Contribution No. 497 from the Forest Biology Division, Canada Department of Agriculture.
[2]Forest Biology Laboratory, Quebec, Que.

few samples which had been oven-dried. Until required for testing, the probes were stored in small, weighed, glass vials equipped with aluminum screw-top lids, and a record was kept of their individual weights. At the conclusion of each test, the probe was removed from the wood, placed in its appropriate vial, the new weight recorded, and then replaced in the wood. It was a simple operation to compute the moisture content of the probes and the corresponding moisture changes in the wood from the recorded data.

The efficacy of the method was not reduced when differences in density occurred between the samples and the probes. In these trials the probes were white birch (sp. gr. 0.55), but it was possible to estimate with reasonable accuracy the moisture in samples of lodgepole pine (sp. gr. 0.47) and aspen (sp. gr. 0.34). However, when greater accuracy is desired, or when the densities of the probe and the material to be measured are substantially different, an estimating equation based on the results of actual experiments should be employed.

Although the technique was developed mainly as a research tool for studying the ecology of fungi in living trees, it has practical application in many other aspects of timber research. It would seem to have considerable potential value as a means of measuring the seasonal moisture status of timber or other biological material in storage, or of felled timber and slash in the forest. The technique is non-destructive in the sense that the measurements of moisture may be repeated at the same point in the sample, indefinitely.

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Ottawa, Canada.

NOTES TO CONTRIBUTORS

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